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ABSTRACT

This volume contains the prepared papers and discussions of a National Academy of Sciences - National Research Council Symposium on the Future of Animals, Cells, Models, and Systems in Research, Development, Education, and Testing. The purpose of the symposium was to examine the past, present, and future contributions of animals to human health and welfare; the present and future use and limitations of cell, tissue and organ cultures; and the application of statistical and computer technology to biomedical research as a substitute for living systems. Titles of presented papers include: (1) The Era of Human Awareness; (2) Experimental Systems: Advantages and Disadvantages; (3) Animal Models; (4) A Legislator's View on Animal Legislation Affecting Biomedical Research; (5) "In Vitro" Systems in Basic Biomedical Research; (6) The Ethics of Biomedical Experimentation; and (7) Root and Branch: Legal Aspects of Biomedical Studies in Man and Other Animals. (MR)

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The Future of Animals, Cells, Models, and Systems in Research, Development, Education, and Testing

The National Research Council

SE 023 431

The Future of Animals, Cells, Models, and Systems in Research, Development, Education, and Testing

Proceedings of a Symposium
Institute of Laboratory Animal Resources
Division of Biological Sciences
Assembly of Life Sciences

NATIONAL ACADEMY OF SCIENCES
WASHINGTON, D.C. 1977

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the Councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by the Report Review Committee, consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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Preface

This volume contains the prepared papers and discussions of a National Research Council–National Academy of Sciences (NAS–NRC) Symposium on the Future of Animals, Cells, Models, and Systems in Research, Development, Education, and Testing. It was held in the National Academy of Sciences Auditorium in Washington, D.C., on October 22 and 23, 1975.

The symposium was organized by a committee of the Institute of Laboratory Animal Resources, Division of Biological Sciences, Assembly of Life Sciences, NAS–NRC. The members of the committee included a concerned lay person, active laboratory bench researchers, a biologist, the dean of a veterinary college, research administrators, and a retired dean of a medical school.

The purpose of the symposium was to examine the past, present, and future contributions of animals to human health and welfare; the present and future use and limitations of cell, tissue and organ cultures; and the application of statistical and computer technology to biomedical research as a substitute for living systems.

The participants invited included the president of a lay organization concerned with animal welfare, a member of Congress, the president of a state university, deans of schools of various health sciences, research scientists, a lawyer, a mathematician, behavioral scientists, professors in medical and veterinary schools, research administrators from government and industry, and physicians. They came from this country, Canada, and abroad.

Formal papers were submitted in advance and invited discussants prepared critiques and summaries after groups of related papers. Four sessions of one-half day each were held in the 2-day period. All meetings were open to the public and press, and discussion was invited from the floor. The often spirited exchanges are found in the proceedings.

The impetus for holding the symposium came because of general public interest in all facets of health, the attention given in the press to use of animals in research, the concern of Congress with the progress of research it has funded over the years, and the hope of groups of citizens concerned with animal welfare that sufficient progress has been made that changes in research project designs might be instituted in the extent and type of use of animals. The latter group spoke first to express their concern. Scientists followed with detailed discussions of the current state of the art in their own disciplines. Many more questions were posed than could possibly be answered in the time.

The key problem considered from many angles was whether intact animals could be eliminated from use in research or their numbers reduced by substituting or expanding the use of newer techniques for *in vitro* studies and computer simulation. Recognition was given to significant research already being done in *in vitro* systems, but it was concluded that certain types of research always will require whole living animals. The proposed newer methods will provide data to supplement that obtained by traditional ones proved by time and when properly used can reduce the number of animals required. The techniques offered as solutions will continue under steady investigation for assessment of their value in the long run as compared to traditional experiments. The exposition of the current use of the methods was excitingly presented.

An area of major agreement was that the dialogue begun in the symposium was extremely useful to all who attended and joined in the frank exchange of ideas and opinions. It was agreed that future research should continue to explore the proper scientific role of *in vitro* and nonliving techniques.

An area of disagreement was over current laws and regulations and whether they were adequate to protect animals, especially endangered species. Currently accepted methods using whole animals for screening drugs for possible human use were challenged as outdated and in need of revision. Scientists using the methods felt not enough was known about how the whole body functions through its feedback mechanisms and interrelation of metabolism between various organs and systems in the process of utilization and breakdown of chemicals to permit substitution of *in vitro* techniques at this time.

Future prospects are encouraging for further adaptation of *in vitro* techniques after exhaustive study and comparison with older ones. Continuation of the dialogue started was recognized as useful and necessary in the future better accurately to disseminate new information and to help assure adherence to the humane objectives of both lay citizens and scientists. It is hoped these deliberations will be helpful to those groups charged with formulation of public policy.

George T. Harrell
Chairman, Organizing Committee

Organizing Committee

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GEORGE T. HARRELL

Introduction

The Symposium on the Future of Animals, Cells, Models, and Systems in Research, Development, Education, and Testing was sponsored by the National Academy of Sciences and National Research Council. These organizations are advisory to government and other agencies on questions that might involve national problems. The Division of Biological Sciences of the Assembly of Life Sciences is a branch of these two organizations. The Institute of Laboratory Animal Resources (ILAR), a smaller branch, has been in operation for over 20 years.

ILAR is particularly interested in the quality of animals, materials, and facilities that are used in animal research and in teaching. It has appointed a number of committees to study many facets of animal problems in which they advise, recommend, persuade, or cajole investigators and institutions in the proper use of animals.

The committees have published a number of technical papers that are essential to proper animal care. The National Academy of Sciences does not itself operate any research programs. ILAR helps the biomedical scientific community through advice only.

The charge to the Organizing Committee from ILAR reflects its main goal, which is to help provide wise, humane, economic, and effective use of animals of all kinds, predominantly in research.

For the past 30 years, since World War II particularly, we have been in a golden age of biomedical research. The explosive growth has been financed not only by private foundations, such as the Rockefeller Institute for the problem of pneumococcal pneumonia early in the century, but also the National Science Foundation, for example, which has funded basic research. The National Institutes of Health and the Armed Forces have also supported basic and applied research.

Information in the basic sciences is absolutely essential, not only for knowledge in its own right, but also in serving as the basis for application to clinical problems. This fact is true both in human and animal diseases.

From the educational point of view, and, having spent most of my professional life as the dean of medical schools, I always speak of education, we are interested in the incorporation into the educational system of the pattern of research thinking. In the training of physicians, the essential intellectual process is problem solving, which can hardly be taught in a better fashion than by observation and participation in properly designed research projects.

On the question of the use of animals, we recognize in the education of medical students and of other health professionals as well that the use of animals in necessary experiments during the educational process provides one of the best opportunities for the early development of a proper professional attitude of compassion. Well-conducted animal experiments help in the difficult task of preventing the development of what we have been accused of producing—callousness in the attitude of medical students. The students come in starry-eyed; they like animals and they like people. We should be sure that these traits and attitudes are retained.

Before World War II, the emphasis in biologic research was largely on the infectious diseases. This emphasis was entirely proper, because these diseases were the great killers of people worldwide. Malaria is probably the most important disease the world has ever known. It has killed more people than any other disease, and what many of us overlook is the fact that malaria has killed predominantly children who have no immunity and no protection from the mosquito vector.

The other great killer, again predominantly in children, is dysentery. Even in some parts of the world today, a high proportion of children born never reach their fifth year, because they die, usually in the first 2 years, of dysentery.

Pneumonia, in the past in this country, was called the "friend" of the aged. This was particularly true of pneumococcal lobar pneumonia, because it killed elderly or debilitated people very quickly. Tuberculosis was the other great killer that we faced early in the century.

With the coming of World War II, considerable emphasis was placed on the approach to prevention, if possible, or treatment of those diseases associated with crowding that would be transmitted by upper respiratory infections. This concern led to the review of what was known about the chemical materials that might kill bacteria and control meningitis, pneumonia, and other infections. This goal was successfully accomplished, and no epidemics, as occurred in World War I, resulted from the crowding of susceptible individuals into camps or subways while bombing was threatened or in progress.

Now, the chief causes of death have changed. They are the chronic illnesses, predominantly heart disease, stroke as a manifestation of atherosclerosis, and cancer. The chief causes of disability with loss of time at work are also chronic illnesses—arthritis, congential defects, the genetically transmitted diseases that reflect metabolic alterations in enzyme patterns, and mental illness, which still is one of our major public health problems.

This latter problem reflects behavioral disturbances. We do not know a great deal about the basic biology of behavior, not only in the human being, but in all animal species. Many of the actions seen, which we tend to interpret as specific to the human being, are not that at all. Sexuality, territoriality, and aggression are seen in most species of animals and can be properly studied in them.

We still have the problem of serious viral diseases transmitted by food, water, close association, or by insects. Other, less serious respiratory viral diseases are a cause of disability. Only a few can be treated by chemotherapy or prevented by vaccines.

In the research on infectious agents, we studied bacteria, viruses, higher parasites, produced vaccines, and developed drugs—the magic bullets that were predicted early in the century with the use of heavy metals. Then we moved to dyes, and the various sulfonamides were developed that were a great help early in World War II.

Later in the war came the recognition of penicillin as an agent to control many bacterial and spirochetal infections. Penicillin is probably the most important drug that has been developed in this century, if not in the history of the world. It has a wide margin of safety and is very effective in acute infections. It is one of the drugs to which bacteria develop drugfastness or nonsusceptibility relatively slowly. Other antibiotics followed.

Then we began to develop drugs that gave symptomatic relief to some degenerative diseases such as arthritis, that would alleviate some types of hypertension, and others that would control outward manifestations of the more serious mental problems. The development of psychotropic drugs—particularly tranquilizers—has resulted not in a very great reduction in the number of hospital beds still devoted to mental illness, but to a considerable shortening in the length of stay of those patients who must be hospitalized.

As a result of this intensive research over the last 40 years, the biomedical community essentially has solved the easiest and most approachable acute human disease problems. But we must face the fact that we still lack the basic biologic information on which to approach the control and cure of these chronic killers and disabling diseases. We just don't know what the mechanisms are that produce most of these chronic illnesses.

Must we work only with the human being in the future, or is it possible to develop models of these types of disease problems in animals, or can *in vitro* techniques be used? This question was the whole basis for this symposium.

In the past few years, increasing public interest has developed in all matters affecting health. Great economic concern has been expressed about the cost of the delivery of health care, which has become one of the three major industries in this country. The press pays attention to nutrition, food additives, and environmental pollution. What are the long-range effects of these materials on people? These chemicals cannot be studied over years in the human being alone, but they can be studied in a much more controlled environment in other species.

What is the effect over the years of exposure to self-administered drugs? How do we anticipate, if it is possible at all, the unexpected side effects of drugs? Reactions are a biologic fact that follow a bell-shaped probability curve. Even with a very important drug, penicillin, some people react violently the first time they are exposed to it.

How do we study these phenomena? We can't do it in the intact human being. It must be done in some other fashion. We must admit we have made very slow progress in recent years in the understanding of stroke, cancer, arthritis, birth defects, and most genetic diseases. We have identified them, but what to do about them is another matter. When you compare the present pace with the dramatic progress made in the thirties and forties in the infectious diseases, you recognize the difficulty of the problems.

Unfortunately, fewer funds are now available for these studies, while the costs of biologic research have increased. Research has become more complex, because the problems are more difficult. We are using more animals of more different species, as we learn about the types of models that can be produced.

As a result, some species, such as certain of the primates, now are being endangered. What do we do about this potential loss? The control of all the variables is impossible in the human being and would be socially unacceptable even if it were scientifically possible. The time is right to reexamine the stage on which biomedical research is conducted.

We hope that this symposium has suggested answers to some of these questions. Others that should be raised include whether some of the long-accepted basic premises on which biomedical research has been evaluated are acceptable now in the light of new instruments and techniques. Such concepts as the LD_{50} when you test a new drug need to be reexamined.

We recognize that in the past animals have contributed enormously to human welfare, but have researchers paid enough attention to the animals themselves and the setting in which they are held while under study? This question is not just one of a humanistic approach, but a scientific one. How does the setting affect the accuracy and the precision of the data that you collect?

Are we using the proper species of animals for our particular research projects? Could we use lower forms than mammalian species to achieve the same results? Are we using too many animals? Could we do with fewer if experiments were better designed? Would a more detailed analysis of data give us more dependable conclusions and reduce our dependence on large numbers of animals? Can we substitute for whole animal experiments *in vitro* techniques by which cells, tissues, and organs can be cultured?

How do we handle the important feedback mechanisms of the intact animal. Are they present in the available *in vitro* methods, or are we losing something critical to the research? Is it possible to substitute computer simulation in biologic experiments, as has been done in studies of engineering, physics, and other fields where graphic displays, of great inherent beauty in their own right, can be produced? In this fashion can you isolate a single variable and study it? Can you account for the basic biologic principle of variability, which is inherent in all living things and must be considered in all biomedical research. Can the feedback mechanism be simulated?

In any event, the Assembly of Life Sciences, through the Institute of Laboratory Animal Resources, appointed an Organizing Committee composed of researchers, teachers, academic and government administrators, and lay members concerned with animal welfare. These people, with backgrounds as physicians, veterinarians, basic scientists, clinicians, and humanitarians, were charged to examine, looking first at the past and then projecting into the future, the present status and trends in research, education, and testing from the scientific, technical, philosophic, ethical, and legal aspects.

The participants were recruited not only from this hemisphere, but also from abroad. They are experts in the fields from which they were chosen.

FRED C. DAVISON

Historical Perspectives of Biomedical Experimentation

History is the essence of innumerable biographies.

Thomas Carlyle, *On History*

The subject "Historical Perspectives of Biomedical Experimentation" is made difficult only by the fact of its size. Any comprehensive treatise on the subject would fill volumes, and any abbreviated version runs the risk of doing less than an adequate job. We are advised to study history in most cases so that we may avoid the mistakes of previous generations. In this case the study recommends itself so that we may continue on a course that has brought untold progress to mankind. An understanding of the past has always been necessary as man has looked to the future in any of his endeavors.

It is certainly appropriate that we undergird ourselves with historical perspectives as we gather to look at the future of animals, cells, models, and systems in research, development, education, and testing. This exercise has been both enlightening and reassuring to me. I have found myself once again caught up in the excitement of great men's accomplishments and struggles. I have been reimpressed by the knowledge that, while there have been high peaks and deep valleys in the progress of man's understanding of himself and of his environment, there has always been an irrepressible concern and curiosity that has led him to the next plateau of knowledge. It has also been of great interest to me to note that, in general, this journey of achievement has seen man's welfare inseparably tied to the animals over which he was given dominion.

Biomedical research, our subject in general, has evolved from and is a part of medicine, and medicine, as it has dealt with both man and animals, is as old as recorded history. Outstanding men throughout the ages have recognized this unity, and it seems that only in professional practice have the concerns between man and animals been separated, while both have continued to be based on the same principles. In the areas of research, preventive medicine, public health, and others, the line of demarcation is in many cases indistinguishable. Whether directed toward man or animals, medicine is concerned utlimately with the acquisition and application of knowledge for the betterment of man. It generally has been so in history. The Egyptians practiced a single medicine, treating man and animals on a comparable level, and their methods were relatively sophisticated for their times. Mosaic Law clearly indicates that the ancient Hebrews recognized the transmissibility of certain diseases from animal to man. The recognition of this interrelationship of disease processes in man and animals flows through recorded history in a remarkable way.

According to early Vedic (Hindu) records (approximately 1500–500 B.C.), man acquired the art of medical practice by observing animals and birds. Veterinary medicine in ancient India was quite advanced for the times. The Greeks studied and taught both human and veterinary medicine and appreciated to a remarkable degree the interrelationship between the two. Also, during the Roman and Byzantine periods, medicine achieved a high level of sophistication. Virgil, Aristotle, Hippocrates, and their followers all diligently studied animal life, including dissection. From these observations they made many conclusions relative to human medicine that are as valid today as when they were formed more than 2000 years ago. Following the fall of the Roman Empire and during the Dark Ages, all arts and sciences declined. Both human and veterinary medicine deteriorated. During this period man began to look upon animals as being so totally different from himself that he was unable to see how the two branches of medicine could possibly have anything in common. As a result, a great but temporary disparity developed between them.

With the Renaissance, medicine began to reappear. In the seventeenth and eighteenth centuries, human medicine was revitalized by men like John Hunter, Marcello Malpighi, Stephen Hales, Robert Hooke, Edward Jenner, and others. Then as now, these men were limited in their work with human beings. Therefore, much of their study was done on animals, and the significant advances were frequently a direct result of animal observations.

Veterinary medicine as a separate practice did not recover from the Dark Ages until a century later. It began its rebirth in the eighteenth century in France.

From that period on, however, progress has been steady and at times spectacular, and we have seen in the field of bioscience a strong series of bridges develop linking man's study of himself and his study of animals. This development has been essential, since the interrelationship between man and animals is intimate, significant, and far-reaching. While man has always been dependent upon animals for food, clothing, work, and companionship, he has also been a victim of their diseases either directly or indirectly.

These diseases, both animal and human, have always influenced the destiny of man. The great animal and human plagues across the centuries have brought famine, death, poverty, and even the downfall of empires. Out of necessity we have learned from our experiences, so that today's knowledge is based on our ability to learn using all of the tools available in science. A major lesson of history is that we must not limit ourselves concerning the availability of those tools if we are to conquer the adversities that face us either at the moment or in the future. This is perhaps the great lesson of the Dark Ages. In our particular struggle, the tools are those of knowledge. They are the tools of the principles of physiology, pharmacology, microbiology, immunology, pathology, genetics, and so on. Historically, these tools have proved to be essentially the same whether in man or animals, and research benefits both regardless of which serves as the primary unit of experimentation.

Perhaps an effective way to flesh out the skeleton of such a cursory historical outline would be by following Thomas Carlyle's suggestion that "history is the essence of innumerable biographies," and to cite in this continuum of biomedical progress the works of a limited number of people—people who best illustrate the single purpose of medicine as a generic enterprise and who prove that the enterprise itself is indeed a composite of many disciplines using many tools. Such a list would include but certainly not be limited to the following:

Aristotle (384–322 B.C.) was a philosopher, but he was also a biologist. He was the son of a physician, and his observations of animals enabled him to found the sciences of physiology, zoology, and comparative anatomy. He concluded that animals, including man, are closely interrelated. In *De Partibus Animalian* he states, "The course of exposition must be first to state the attributes common to whole groups of animals and then to attempt to give their explanation."

Galen (130–200 A.D.) was a physician, anatomist, physiologist, and philosopher. He was the founder of experimental physiology. He was an avid dissector, and his investigations on the anatomy of monkeys, pigs, and dogs were complete and accurate and resulted in a greatly improved understanding of the human body. He did not dissect human bodies, but his studies with animals were designed for application to human medicine.

William Harvey (1578–1657) was a physician. He dissected every animal, vertebrate or invertebrate, that he could obtain and studied their circulatory systems. He studied the pulse of man and animals and concluded that contraction, not expansion, was the heart's most important function. His studies, mostly in animals, unraveled the true nature of the circulatory system and indeed were some of the major biomedical contributions of all time. He probably did more than any man of his time to establish that observation and experiment were as respectable as classical scholarship.

Robert Boyle (1627–1691), who was a chemist and physicist and is known as the "Father of Chemistry," and *Robert Hooke* (1635–1703), who was a philosopher, confirmed with experiments on mice that some substance in the air was necessary for life.

John Hunter (1728–1793) was one of Britain's most distinguished and influential medical teachers and surgeons. He made many diverse contributions in comparative medicine, such as the discovery of the air sac system in birds, the production of heat by animals, that fish have organs of hearing, and the structure of whales.

Edward Jenner (1749–1823) was a physician. He pursued the popular folk belief around Gloucester, England, that an infection of cowpox (vaccinia) in humans would protect against smallpox (variola). He, of course, proved the immunological relationship between the two. From observations of man, he determined that cows were involved in the control of smallpox and made the first successful use of an avirulent material to produce immunity to disease.

Louis Pasteur (1822–1895) was a chemist, but his greatest contributions were in immunology and microbiology. While studying the microorganisms that cause fowl cholera, he accidently discovered that when the attenuated organism was injected into healthy chickens, they became resistant to subsequent virulent doses of the organism. Further work with anthrax in sheep supported his immunization theory (1881). He next studied rabies. After long and diligent efforts, he successfully cultivated the virus in rabbits, attenuated it, and then immunized dogs against rabies. He was reluctant to use the newly developed vaccine in man, but in 1885 he injected a boy who had been severely mauled about the head by a rabid dog. Immunity developed before the onset of the fatal disease, thus saving the boy's life. A great step had been made. His work with animals revolutionized human and veterinary medicine.

Robert Koch (1843–1910) was a bacteriologist. He was the first to prove that a specific microorganism (anthrax) was the etiological agent for a specific disease, thus establishing the "germ theory" of disease. Likewise, Koch was the first to isolate the tuberculosis organism. He discovered that tuberculosis in man and cattle was the same disease and that anthrax in man and animals was identical. Only after this discovery did the void that had developed between human medicine and veterinary medicine disappear. Koch made major contributions to bacteriology, many of which were heavily dependent upon his studies of animal disease.

Koch, Pasteur, and other investigators of that era began to realize that lessons learned from studying animal disease were necessary and that they could be applied to problems associated with man. From this time on the pace of progress quickened.

Theobald Smith (1851–1934), a physician, and two veterinarians, *F. L. Kilborne* (1858–1936) and *Cooper Curtice* (1856–1939), were in 1893 the first to conclusively demonstrate the transmission of a disease-causing agent by an arthropod vector—the relationship of Texas fever and the tick as a vector (animal-to-animal). Their work set the precedent for studies that ultimately helped control malaria (man-to-man), yellow fever (animal-to-man), and rickettsial spotted fever (animal-to-man).

Friedrich Löffler (1852–1915) and *P. Frosch* (1860–1928) were German medical scientists who in 1897 recognized the first viral disease. They were working with foot-and-mouth disease, a disease limited to cloven-footed animals.

Sir William Osler (1849–1919) was a physician and a pioneer in comparative medicine. He stated, "There is only one Medicine," and his innumerable contributions to the knowledge of disease saved animals as well as man.

Frederick G. Banting (1891–1941) and *Charles H. Best* (1899–) discovered insulin in the early 1920's while working with dogs. These findings led to a treatment for diabetics, and millions of human lives have been extended.

Gerhard Domagk (1895–1964) was a pathologist–bacteriologist, who, while working with experimental streptococcal infection in mice, discovered the antibacterial effect of prontosil, the first of the sulfonamide drugs. This was the first major chemotherapeutic development in the control of infectious diseases. In retrospect, it was indeed fortunate that Domagk was working with mice rather than using *in vitro* techniques. It was later found that prontosil was inactive *in vitro*. Its effectiveness was dependent upon the release within the body tissues of sulfanilamide. Otherwise, the discovery and use of chemotherapeutic agents could have been delayed for years.

From this time forward, the pace of accomplishment becomes dizzying, and the list of individual achievements defies cataloging. Indeed, credit to all of those who deserve mention has filled encyclopedias.

If we deviate from the plan of following individual contributions, we can easily substitute other perspectives that group achievements by specific areas. These advances can also be related historically to the use of animals, cells, models, or systems. In each case, however, the use of animals would seem to dominate. For example, in pharmacology there can be little doubt that the traditional and basic procedure of animal experimentation has been central in the development of drugs used in the treatment of diseases of known etiology—drugs such as antibiotics, antiparasitics, antiallergenics, and others. It has also led to the development and validation of drugs useful in the treatment of diseases of unknown etiology—drugs such as antiinflammatories, pain relievers, and drugs for nonspecific disorders such as heart and renal diseases. The whole field of nutrition has its foundation firmly rooted in animal experimentation, which has led to the discovery of diet essentials, the understanding of deficiency diseases, the interrelationship of diet and cardiovascular disease, and such recent mass programs of disease prevention as fluoridation. The literature shows that advanced surgical techniques (cardiac surgery and organ transplants to name but two of the more recent and glamorous, which will in their time move to the classification of routine), the control and management of hemorrhagic shock, the development and use of nuclear medicine, and even the conquest of space would not have been possible under concepts of research different from those that have evolved from centuries of efforts using animals as the basis for experimentation and observation.

It is remarkable to note that because of successes in biomedical research—particularly in the field of infectious diseases—our country exists almost as an island in a sea of what are to us exotic diseases. The fearful plagues have been brought under control in our animal population. Equal advances have been made in those scourges of the human population—diseases that were real threats to most of us as children. This is an enviable but also dangerous position—for our success has left us vulnerable, if we become careless or complacent, to those very diseases that we have pushed beyond our borders. Our work can never be finished. We can never rest on our laurels. I doubt that any of us would be willing to return to a society in which smallpox, bubonic plague, cholera, typhoid fever, diphtheria, scarlet fever, typhus, or polio were unchecked, or to an economy in which our supply of animal protein could be wiped out at any time from an equal number of infectious animal diseases. And yet we could.

While background work for this brief paper has been enjoyable and, as I noted earlier, reassuring, it has also been sobering. History shows that those who have worked to advance the science of medicine have always had their critics and that there have always been those in opposition. Alive in much of this opposition has been the question of the use of animals in experimentation and the quest for alternate methods. The topic of my paper has caused me to think primarily from a perspective of the past; however, it has led me to several conclusions that also speak to the present and to the future.

First, if in the world of politics the price of freedom is eternal vigilance, the price of advancement and even security in the world of bioscience is a constant research effort—an effort in which all resources are wisely used and in which the researcher must be free to direct his inquiries and to choose his paths and his tools with only a minimum of external control.

Second, recent successes have been so commonplace that our greatest danger may be that of taking them for granted.

Third, we may turn our backs, for any one of several reasons, on a process that has been remarkably effective. While I agree that every new and alternate method must be incorporated, we must not forget that the body and life are enormously complex and that, even though we know a great deal, there is, in all likelihood, far more that we do not know about life processes. Body functions consist of millions of actions and reactions. Each is dependent upon many others, and it is highly unlikely that man will ever be able to simulate all of the complexities of the body with alternate methods. For quality-control purposes, alternate methods have always had to be checked with the intact animal.

Fourth, the following question must be asked today, as it has been throughout the history of biomedical research: how many people would be willing to take a new drug or try a new vaccine, have a new surgical procedure performed, or even use a new cosmetic if it had not been first tested on an intact biological system?

Fifth, I have been continually impressed by the fact that so much of what we know that is vital has come not from studies planned to answer specific questions. It has come as fortuitous information recognized by astute observers who understood thoroughly the animals with which they were working.

Sixth, I am left with the final conclusion that to abolish the use of animals in teaching and research would fly in the face of the lessons of history and be far more inhumane to society, both man and animals, than to encourage their reasonable, humane, and justifiable use.

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CHRISTINE G. STEVENS

Humane Perspectives

The elegant architectural drawings of the Italian Renaissance can give a long, romantic perspective ending at a vanishing point in the far distance or a bold, immediate perspective in which a great building rises sharply in front of us and the perspective falls away so rapidly that all but the foreground is dwarfed. If we translate visual perspective into time, the choice between these two manners is open as well. We may emphasize the lengthy debate stretching back through a misty middle distance to Queen Victoria's reign, when sharp criticism of cruelty to experimental animals began, with the horrors of a series of major operations on unanesthetized horses in the veterinary school at Alfort, France (1). Then, if we take the long view on attitudes towards animals, the perspective leads back till the mist turns thicker and foggier among the ancients. Alternatively, we may be transfixed by the overriding importance of the present and dismiss the past with a few sketchy lines.

The spirit of our time is against authoritarianism, against the image of the cruel tyrant who rules his subjects by force and compels them to undergo hardships. Cruel animal experimentation is thus contrary to the spirit of the times. But animal experimentation should not and need not be cruel.

An example of a cruel experiment I noticed in looking through the *Journal of Drug Research*, vol. 6, no. 3, December 1974 (2), published in Cairo, Egypt, describes how the development of peptic ulcers is:

> executed through the extensive triggering of the emotion of fear. Prolonged forcible immobilisation of animal, the crippling panic of helplessness will release the reins of the two autonomic components. . . . The first for the induction of neurogenic gastric

> ulcerations was made by squeezing the animal into a tight wooden box (3). Although ulcer did occur, their size and number did not provide a convenient statistical value. This could be explained by the ability of the animal to move the limbs slightly. In this study a more severe type of panic stricken struggling animal was seeked. Forcible fixation with overwhelming muscle contraction with all the autonomic fear syndrome are thus fulfilled in order to obtain the appreciable pathologic lesion. . . . The animal was layed supine on a wooden board with fixation hooks at the four corners. The four limbs were tied without excessive tension to avoid respiratory hindrance. This type of fixation compelled the animal to try hard, but in vain, to fight for release. The sympathetic hyper-excitability was manifest by tachycardia, tachypnea and pupillary dilatation. The tail of the animal was insistently wiggling and showed vasculo-constriction. The rat, although pitied, was left in its sufferings and agony for 24 hours. After the lapse of this period, the animal was sacrificed by cerebral destruction and disemboweled. [*sic*]

What could more precisely exemplify the spirit of authoritarianism than wilfully inducing such extreme fear in small defenseless animals? Yet this took place in a country that 5,000 years ago perfectly mummified and wrapped in linen bands in sculptural harmony the sacred ibis, symbol of the god of science. Such an ibis may be seen at the Egyptian Embassy, in Washington, D.C.

So when we try to see a humane perspective, we have to admit that in some respects we may be looking through the wrong end of the opera glasses.

But if we shorten our perspective, if we look at the past few years in our own country and in the Western European democracies, we see that the darkest side of human nature, that most desperately in need of control, seems to be increasingly subject to regulation in animal experiments.

A resolution passed at the 1975 symposium of the International Committee on Laboratory Animals* calls for legislation regulating animal experimentation in every country that uses laboratory animals—quite a contrast from the early 1960's, which saw such flat, solid, seemingly unbreakable opposition in the American scientific community to mandatory humane requirements.† New laws have been passed in the 1970's in Finland, the Netherlands, West Germany, and Austria that seek to improve the lot of experimental animals, and I would note that it is in new laws and proposals that requirements are found in some cases that alternatives to laboratory animals be used where possible.

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* VI Symposium of the International Committee on Laboratory Animals, July 9–11, 1975, Thessaloniki, Greece.

† Such opposition included actual suppression of a report prepared by the Department of Health, Education, and Welfare, entitled "The Care and Management of Laboratory Animals Used in the Programs of the Department of Health, Education, and Welfare," Division of Operations Analysis, Office of the Comptroller, Office of the Secretary, January 1966. The report, published copies of which were locked in a storeroom, contained documented material critical of the treatment of laboratory animals by National Institutes of Health grantees. For example, on page 131, a summary of Animal Welfare Institute criticisms appears as follows:

There can be no doubt that the right of laboratory animals to protection against cruelty, whether sadistic or nonsadistic, from neglect, from inadequate quarters, hunger, thirst, and fear, is now recognized, and that it is further recognized that self-policing to ensure these rights is not acceptable.

There are those who have denied that animals have rights, but they have already been overtaken and left in a rather dim, reactionary posture by some solid legal thinking on the rights of trees and streams and other natural entities (4) and the way in which the sustaining of these rights benefits us all—both people and other animals.

When Darwin showed that we were 1 among nearly 200 other species of primates and 12,000 to 15,000 species of mammals, he laid the groundwork for a different attitude toward our fellow mammals. It was no accident that he played a leading part in obtaining the first law to regulate animal experimentation, the British Cruelty to Animals Act of 1876. He said the thought of painful experiments made him feel sick and kept him awake at night (5).

But, of course, it is no guarantee of kindness to be recognized as man's fellow mammal, bird or other creature, given the ferocious cruelty with which fellow men have so often been treated by one another. It is important to give recognition to the value of each individual animal, to its ability to feel and to suffer, in order to avoid

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(a) Cages Too Small for Animals to Stand or Lie in Normal Positions:
Institutions visited—8 (7 grantees, one intramural)
Species affected—dogs, rabbits, monkeys

(b) Failure to Administer Pain-Relieving Drugs After Surgery
Institutions visited—3 (grantees)
Species affected—dogs, and all laboratory animals used

(c) Failure to Destroy Suffering, Moribund Animals:
Institutions visited—2 (grantees)
Species affected—dogs

(d) Failure to Supervise Animals After Surgery:
Institutions visited—3 (grantees)
Species affected—dogs

(e) Failure to Provide a Comfortable Resting Place for Animals:
Institutions visited—3 (grantees)
Species affected—cats, raccoons, dogs, other species

(f) Repeated Use of the Same Animal for Painful Procedures:
Institutions visited—1 (grantee)
Species affected—dogs

(g) Failure to Provide Water:
Institutions visited—4 (grantees)
Species affected—monkeys, rabbits, dogs, cats, guinea pigs.

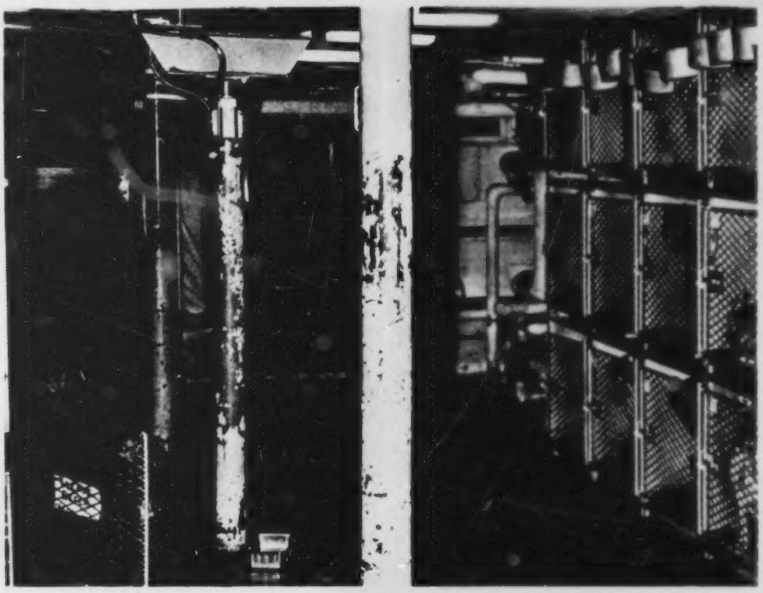

FIGURE 1 Dog room, University of Minnesota, January 1966.

self-righteous abuse of animals by the ignorant, who may otherwise regard them as mere things. But beyond that, in considering the situation of laboratory animals, so similar to that of human prisoners, the massive problem of human cruelty and callousness must be tackled. It has never yet undergone any serious scientific scrutiny.

The scandals of the nursing homes, full of neglected and abused old people, exemplify one aspect of this problem. The recently published *Alexander Dolgun's Story: An American in the Gulag* (6) constantly brings to mind parallels with experimental animals as the reader visualizes the long corridors of cell doors—like the long rows of cages—the deliberate sleep deprivation for the purpose of obtaining desired information ("Talk, and you'll get sleep.") (p. 80)—like sleep deprivation studies in animal laboratories, also conducted for the purpose of obtaining information—even to the brief periods of exercise and removal from the cell once a day. "Around seven . . . the guard . . . takes you down some steps and out into the yard for the exercise period . . . I have fifteen minutes for fresh air." (p. 52) But, as the pressure was stepped up, the victim was moved to the most dreaded of the prisons, Sukhanovka:

> I was never taken outside. For that whole period in Sukhanovka, I never saw the sky, although by the end of March, the smell of pines coming in through the window when it was open for those few precious minutes each morning gave me visions of an outside world and gave me a little hope somehow.

These lines, speaking of the sense of smell, which is such a feeble sense in man as compared with the dog, bring home the cruelty of the deprivation of outdoor exercise for that species in particular.

There certainly is no broad-scale intention on the part of the American scientific community to "break the spirit" of stray dogs, the so-called random-source dogs, it purchases from pounds and dog dealers, by shutting them all up in cages and never letting them out again. But the fact is that these creatures, whose olfactory capabilities are infinitely superior to ours and whose delight in running is based on a long evolutionary history, are being stubbornly subjected *for no purpose at all* to treatment reserved for suspected spies in the cruelest of the Soviet prisons in 1948 under Stalin! Clearly, this makes no sense, and the obstinacy with which certain elements of the scientific community has stuck to its determination to continue this unjustified deprivation makes suspect any claim of serious responsibility for laboratory animals by the scientific community as a whole.

Since no one claims that science will be advanced by routinely keeping dogs uncomfortable rather than comfortable, why should the public believe claims of many institutions that they never cause pain to experimental animals even when conducting research? A 1973 analysis of reports to the U.S. Department of Agriculture, under the Animal Welfare Act of 1970, shows that the majority of institutions reporting indicated no pain was caused that was not relieved by anesthetic, analgesic, and tranquilizing drugs. The only large-scale exceptions given in these reports were based on government drug-testing requirements cited by manufacturers of pharmaceuticals who reported, for example, on use of "the writhing test"* to measure the potency of analgesics. (Writhing, of course, means writhing in agony—a standard test that should not be tolerated in any civilized country.)

Anyone who has visited laboratory animal rooms and talked with those in charge knows that much pain and fear goes unrelieved, whether by drugs, kindness, or intelligent planning, and that infliction of unrelieved suffering is not by any means confined to the drug industry.

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* Warner-Lambert Research Institute, Morris Plains, N.J., reported in 1973: "Approximately 100 experiments using 5000 mice are conducted yearly in evaluating the analgesic properties of potential agents. Mice are given phenylquinone, i.p., and after a few minutes begin to writhe and stretch their abdomen from injection of this irritant. Other techniques required to induce pain in rodents (i.e., hot plate, tail flick) are used as secondary procedures in analgesic development" (7).

The temper of the times is as intolerant of "cover-ups" as it is of authoritarianism, and, viewing past, present, and future in perspective, I recommend strongly that all scientific institutions using animals thoroughly review and accurately report on the means they are now using to prevent poorly planned research, testing or education that inflicts suffering, either physical or mental, on animals from being carried out. If they find the means faulty or if they find that they have violated Public Law 91-579 and submitted erroneous reports to the Congress through the U.S. Department of Agriculture, immediate action should be taken to end both the laxity and the veiling of unpleasant facts from the public.

Take, as an example, the University of Wisconsin. The 1974 report for the Primate Center's operation in 1973 shows "approximate number used: 600 primates" with none under the "pain and stress" category (8). The 1973 report for operation in 1972 shows a total of 1,302 primates used by all departments within the University of Wisconsin with none under the "pain and stress" category (9). In an interview in *Psychology Today*, April 1973 (10), Dr. Harry Harlow, asked how he creates a depressed state in monkeys, replied, "We put them in a small closed chamber shaped like an inverted triangle—a narrow base and a wider top. You might say it is a modified form of sadism . . . the animals are denied motion play. They just cannot move about." Dr. Harlow goes on to say that the triangle cage "really isn't a sadistic device," but Carol Travis, his interlocutor, asks, "Isn't inducing mental stress sadistic?" and is given Dr. Harlow's rationale—a rationale that is quite legal. But there can be little doubt that a procedure described in this manner by its author ought to appear in the annual report of the University of Wisconsin. The Congress, to whom the final report is submitted, cannot be expected to leaf through journals to find out whether or not it is accurate.

Another experiment, this one reported to the Animal and Plant Health Inspection Service by the Albert Einstein Medical Center, in Philadelphia, describes another procedure that must have caused great mental suffering: "To condition cats by swimming for subsequent experiments on the heart. . . . The cat will start swimming in a tank filled with lukewarm water and under supervision at short periods (five minutes at a time) up to 45 minutes, three times daily. Since these studies require utmost physical performance by the cat, general anesthetics or sedatives are not used. At the end of four months, the cat will be sacrificed and the heart will be weighed (including right and left ventricles separately). The studies will be performed on isolated papillary muscles of the right ventricle in the myograph setup" (11).

Another experiment in which physical pain plays a minor part while mental suffering plays the major part is described as follows by the ITT Research Institute of Chicago: "Seven hours a day, seven days a week, twenty monkeys individually caged, are exposed to risk of electrical shock. Lever pressing response every 15 seconds avoids shock completely. Failure to respond causes delivery at a rate of 100 per minute, repetitive, single, 60 cycle, 2 milliamperes, 50 millisecond duration shocks to the feet through a grid floor of ¾ inch diameter tubing." A mechanism is described to prevent continuance if a monkey is "incapacitated and fails to respond within a few minutes." The statement indicates, "It is estimated an average monkey receives 50 shocks per day" (12).

An analysis of types of painful experiments from the 1973 reports of registered research facilities to the Animal and Plant Health Inspection Service shows many of them being conducted in psychology departments.

Thus we have an indication that a large proportion of animal suffering in laboratories is not, as is commonly assumed, aimed at alleviation of human suffering by finding cures or means of preventing diseases. Strong representations have been made on this subject by the World Federation for the Protection of Animals. Efforts to focus on feasible substitutes for test or experimental animals are being made by the Fund for Replacement of Animals in Medical Experiments (FRAME), whose ATLA *Abstracts* (13) is subscribed to by universities and research institutions throughout the world and should be subscribed to as a matter of course by all medical libraries. Published twice a year, it consists of abstracts from the international scientific literature in 16 fields in which tissue or organ cultures, computers, mathematical models, or other methods are used instead of animals. The purpose is to encourage investigators to consider these methods, use them wherever possible, and develop further non-animal-using approaches to research and testing. FRAME takes no part in legislative action.

Indeed, there has been no legislative action relating to laboratory animals for a long time in the United Kingdom, despite the fact that an extremely competent and comprehensive governmental "Report of the Departmental Committee on Experiments on Animals," commonly called "The Littlewood Report" after its chairman, Sir Sydney Littlewood, recommended such action in 1965. In the "Summary of Conclusions and Recommendations," 15 general findings are listed, the first two of which are basic to any broad view of humane perspectives: "1) Animal experiment is a complex and highly specialised subject. It is also a moral and social problem of the first magnitude and one that does not exclusively concern the expert. 2) There is general recognition that anyone who makes use of an animal in research incurs a moral responsibility to justify his action and a duty to limit pain and give proper care." Number 10 is also worthy of note: "There is no scientific evidence that any single vertebrate species is more sensitive to pain than another" (14).

Some years have passed since the National Society for Medical Research flooded the news media with assertions that laboratory animals were "more pampered than pets." Figure 1 shows one of the University of Minnesota's dog rooms not unlike those of a good many other medical schools during the pampered-as-pets propaganda period, so there will be no mistake about the quality of the doublethink.

Even today, with the Animal Welfare Act in effect, it would be legal to put small dogs into cages like those at the University of Minnesota and leave them there for years. There is a long way to go even in the relatively noncontroversial matter of care and housing of laboratory animals. We are only now seriously entering the phase of insistence on use of the excellent array of drugs that effectively alleviate or prevent pain and fear. We are only beginning to be serious about using substitutes for laboratory animals, about designing experiments more humanely to avoid physical and mental suffering, and about designing experiments to reduce rather than expand the numbers of animals for any given intervention, especially if it is likely to cause suffering.

If this symposium advances the use of substitutes it will be a first sign that organized science is prepared to do actual work to help laboratory animals. If, on the other hand, as has been charged by United Action for Animals, the symposium is intended to retard such progress (15); if it falls into the category of some of the National Academy of Sciences committee work described in *The Brain Bank of America* (16), in which Philip Boffey documents a variety of instances of how government agencies and commercial interests use the Academy to head off potential criticism, then the confrontation, the division that the Animal Welfare Institute has always sought to avoid, will be sharpened and given a new life. That is the perspective of the immediate future: Will investigators, veterinarians, and administrators stop fighting simple decency in the care, maintenance, and protection from suffering of laboratory animals? Will the pharmaceutical industry, the medical schools, and the government agencies that use animals be willing to take the animals' welfare seriously? Or will there be a new round of angry attacks on one side and stonewalling on the other?

Glancing over a breakdown of 713 form letters (Animal Welfare Institute unpublished data), some of them mere Xerox copies without any address at all, sent in to the Department of Agriculture opposing any release of laboratory dogs from cages, one cannot rule out a continuation of the attitude that has made the American Medical Association's reputation what it is.

If confrontation it is to be, then the attitude toward animals born of the environmental movement and the new knowledge of animal behavior and the capacities of animals in nature revealed through the past 10 years' observations of mammals will be joined on the side of what the American Medical Association likes calling "humaniacs." I hope that battle royal will never have to take place.

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ROBERT L. HUMMER

The Era of Humane Awareness

The majority of the informed citizens of the world are most appreciative of the myriads of advances made by biomedical scientists. They view these advances as being in the interest of the human and animal populations. This same segment of the human population, however, would raise many questions about the ethics of the scientific community if it were aware of the inadequate orientation afforded many of the new or young investigators in the area of proper care, handling, and utilization of experimental animals. This is especially germane as regards to the prevention and alleviation of pain. Additionally, all concerned citizens would question the ethics of the scientific community if it were aware of the limited but existing pressures to make new and sensational discoveries and to publish experiences by some investigators. Illustrations of the latter were published in 1974 in *Science* under the titles, "The Sloan-Kettering Affair: A Story Without a Hero" (1) and "The Sloan-Kettering Affair: An Uneasy Resolution" (2).

The first recorded attempt involving the use of live animals for research was by Erasistratis in Alexandria, Egypt, in 300 B.C. He placed birds in closed containers and withheld food to see if he could discover what happened to the body humors that were lost. Since this time man and his animals have become mutually dependent upon one another, and today man's indebtedness to lower creatures is beyond description (3).

The history of humane legislation dates back less than 200 years. In the year 1800, Sir W. Pulteney attempted to have legislation enacted in England that would have outlawed bullbaiting. It was not until 1822, however, that the British Parliament passed the first law in the history of the world recognizing the rights of animals and compelling humane care for dumb animals (4).

During the centuries since the first recorded use of an animal in research and the enactment of the first humane law, history is replete with indications for additional laws and regulations designed to protect the rights of all living creatures. The passage of the Animal Welfare Act of 1970 as an amendment to PL 89-544 by the Congress of the United States was the most significant legislation in this field in world history.

COMPASSION

It should be obvious to all concerned, nonetheless, that the mere enactment of laws will not provide positive assurance that every experimental animal will be the recipient of all creature comforts they are entitled to receive. Some investigators labor under the fallacy that animals are much less sensitive to pain and mistreatment, and so forth, than humans under similar circumstances. Obviously such individuals have not considered the true definition of the word "compassion." Webster defines compassion as a "deep feeling for and understanding of misery or suffering and the concomitant desire to promote its alleviation."

In view of the wide variety of anesthetics, analgesics, and tranquilizers available today, there is no justification for an experimental animal to experience unnecessary pain. It is the legal (5) and moral responsibility of the investigator to assure that appropriate medication is prescribed and administered.

One example illustrates the irresponsibility of an investigator and his absolute disregard for the application of the full meaning of the word "compassion." In a training program involving the use of chimpanzees, electrical shocks were used as a means of negative reinforcement. One animal required considerable reinforcing, with the result that the skin and underlying tissues over the heel of one foot were extensively damaged—so extensively that skin grafting had to be resorted to in order that the wound could be healed. Investigators of this type are callous and lack both empathy and compassion. Regardless of the quality of research being performed by such individuals, they should be refused the use of animals.

Instances of this kind are the exception. However, the tendency to repeat inhumane acts could very well be an established part of an investigator's psyche. Indeed, such apparently was the feeling of this investigator who insisted that his chimpanzees would remain in the training chair constantly. Until higher authority intervened, the investigator to whom we have alluded continued his inhumane acts.

Countless statements support the criticisms by antivivisectionists and humanitarians that experimental animals are subjected to unnecessary pain. Two such publications are entitled *Animal Models of Agony and Death: The Veterinary Killings* (6) and *The Death Sciences in Veterinary Research and Education* (7). Admittedly some reports are exaggerated, misinterpreted, or published out of context. Available information, however, indicates that the need for a reappraisal by each biomedical investigator of the manner in which his animals are treated is long overdue. It can be anticipated that until such time as all experimental animals receive the humane consideration to which they are entitled, concerned citizens will continue to plague the scientific community in the name of decency and as defenders of the "animal's rights." Admittedly great progress has been made, but much more remains to be accomplished.

PROTOCOL REVIEW COMMITTEE

Generally speaking, veterinarians are fully cognizant of the word "compassion" and apply its meaning to their daily routine within a laboratory animal environment. But there are instances when they are prevented from applying their personal feelings and professional judgment. This happens when a scientific procedure has been approved by the institutional protocol review committee of a given institution, in which too frequently the veterinarian is not afforded membership. In order to upgrade all aspects of biomedical research from a humanistic point of view, the American Humane Association urges that action at the federal level, as well as the laboratory level, be initiated to assure that veterinarians qualified in laboratory animal medicine are included as voting members of all protocol review committees. Furthermore, when in a veterinarian's judgment some of the experimental procedures included in the protocol are cruel and an official impasse is reached, it is urged that at least two laboratory animal medicine veterinarians be engaged as consultants to review the controversial matter. This mechanism will prevent the occurrence of many, if not all, of the acts of cruelty allegedly occurring in the guise of science (8).

TECHNICAL ADVANCEMENT

Forty years ago numerous surgical procedures were performed on farm animals, especially without the advantage of an anesthetic. Brute force and mechanical restraint were used to subdue them. Today, as a result of the development of tranquilizers and the refinement of anesthetics and the perfection of their use, major routine barnyard surgery has practically been eliminated. The animals are now transported to a hospital for large animals, where proper facilities are available to render the animal insensitive to pain and subjected to a minimum amount of airborne or surface bacterial contaminants.

It has only been within the past 15–20 years, however, that the husbandry technology and refinement of procedures used within most of the biomedical research laboratories have also been remarkably improved. All of this reflects the state of the art today, and documents an almost complete reversal of the attitude of laboratory animal colony management.

Only a few years prior to the enactment of PL 89-544, the animal housing facilities in many institutions, as concerned sanitation and creature comforts, were deplorable and an utter disgrace to the scientific community. Why did these conditions exist? In some instances it can be postulated that the business manager determined how the limited research funds would be allocated. Thus, because of his extremely limited knowledge of animal husbandry and the impact it exerted on the health and well-being of the experimental animals, a miserly disproportionate share of funds was designated for the total animal program. In other instances, there was ignorance and/or callousness on the part of the investigator.

Conditions of this kind were recognized by many of the scientific community but were largely ignored by them until the late 1950's when humane groups, led primarily by Mr. Rutherford Phillips and in cooperation with leaders in humane research animal care such as Drs. Sigmund Rich, Bennett Cohen, Charles Durbin, Mrs. Christine Stevens, and Mr. Berton Hill, became active in promoting federal legislation. The objective of this effort was to assure that at least minimum creature comfort would be provided for most of the species used in biomedical research.

The furor, allegations, and counter charges that resulted from these early legislative proposals alerted the general public to the deplorable conditions that existed at that time. The result of the total effort of many concerned citizens, humane groups, and members of the scientific community during the ensuing years culminated in the passage of the Animal Welfare Act of 1966, which was amended in 1970. This achievement has been responsible for dramatic improvements of the physical plants, as well as the quality of animal care and handling in research, educational, and industrial animal colonies. It is a sad commentary, however, that the passage of a federal law was required to achieve these results. The above notwithstanding, some colleges and research institutions were providing adequate facilities and care for their research animals several years prior to the enactment of PL 89-544.

With the awareness of the conditions of the past, an assessment of the present situation should be made. Since the beginning of the use of animals in research, there have been some individuals who violently oppose the use of animals for either research or teaching purposes. The American Humane Association does not entirely share these strong sentiments. In connection with the scientific use of animals, it is possible to conceive of several ethical positions. They differ mostly in one aspect, that is, the relative value placed upon human as opposed to lower animal welfare. The position of the great majority of informed persons is that the humane use of lower animals to increase knowledge and to achieve practical advancement in medicine, agriculture, animal husbandry, and the like is highly ethical (9).

The American Humane Association is fully cognizant and appreciative of the many advances in medicine, surgery, husbandry, nutrition, and the like, applicable both to the human animal and to all other animals, that have resulted from these research efforts. It does not seek to abolish any specific use of animals for the betterment of mankind or other animals. It is solely concerned that cruelty and mistreatment be prevented. The American Humane Association considers the use of animals in biomedical research from a broad and positive viewpoint. It urges that models or species of animals to be used be selected on the basis of sound scientific and economic reasoning and that the experiments will provide scientific data unavailable through other means. The American Humane Association is not antivivisection or antiresearch, but it has the philosophy that all animals have their God-given rights and that the investigator must acknowledge his responsibility to assure that these "rights" are not violated. In other words, so long as the spirit and letter of the law of PL 89-544 as amended by the Animal Welfare Act of 1970 are fulfilled, the American Humane Association would not object to the proper and humane use of animals in biomedical research. Violation of the provisions of these laws, however, will not be countenanced when such violations result in unnecessary suffering by the animals whose bodies are being used as surrogates for mankind and ultimately for his betterment (8).

AWARENESS OF FUTURE OBJECTIVES

It is common knowledge that today millions of unwanted dogs and cats are put to death annually in humane agency and municipal animal *shelters. The large majority of these animals were brought onto this* earth by irresponsible animal owners. Neither the humanitarian nor the municipal animal warden enjoys functioning as an executioner. Nevertheless, both groups have accepted this task with the belief that at least the animals will be killed in a dignified and humane manner, rather than be dragged to death by the wheel of a fast-moving truck.

Some individuals who are associated with research and aware of this aspect of animal control realize that some of the dogs and cats that are being killed could serve a useful purpose for their fellow animals and/or mankind. Reference is made to the use of specially selected dogs and cats for biomedical research in nonsurvival procedures.

Numerous communities have so-called pound-seizure laws. It would seem appropriate in this enlightened age that such laws should be reworded so that animals can only be used for nonsurvival type studies when anesthetized.

FACTUAL REPORTING

Too frequently anyone opposed to a given subject will distort facts to challenge the opposition. There is evidence to indicate that such has occurred within the scientific community, as well as against its members, by those opposed to a specific research effort. In the name of propriety and fairness, it is urged that scientific reports reflect the true events as they occurred and likewise that those humanitarians opposed to a given effort refrain from quoting scientific data out of context.

CONCLUSIONS

In conclusion, many humanitarians are appreciative of the advances made in human and animal medicine and surgery by the biomedical community. The same individuals, however, are adamant that to cause needless pain and suffering by research animals must be prevented.

Good biomedical research data are the end product of logical thinking in the preparation of the experimental protocol, keen observation, and proper selection and care of the animal models.

It is suggested that all concerned ponder over the admonition of Michael Fryer, founder and president of the Crusade Against All Cruelty To Animals, when, following the defeat in 1973 of an amendment to the British Anti-Cruelty Act of 1876, he is reported as saying, "We do our case more good, whatever the aspect under consideration—by facing facts squarely" (10).

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DISCUSSION

FREE: To my recollection, the leadership in the development of laboratory animal legislation came from the Animal Welfare Institute and the Humane Society of the United States. I do not understand why compassion cannot begin at home, because the veterinarians who are our friends and the animals' friends will have and do have jurisdiction over the design of experiments. I don't know whether that sense of compassion can be well-developed if animals—dogs in particular—are used in some schools of veterinary medicine for multiple operations in surgery.

An animal is brought in, he is operated on for one thing, he recovers, then he is operated on for another and another until he is finally worn down. This, Dr. Hummer, is certainly in line with what you were saying. If most of these are random-type dogs, there should be nonsurvival procedures. They are not in many schools of veterinary medicine.

DAVISON: I think you are dealing with people who enter veterinary school largely with an attitude that is one of strong interest in animal welfare to start with.

I think this colors much of what goes on. I recognize the fact that multiple operations are done. I also know in my own experience in our own school that great care was taken. Whether you wish to expose more animals to fewer operations, I think as much as anything else, it comes back to care for each one of those surgical procedures.

THURSTON: I would like to call your attention to the fact that research grants are offered for all types of experiments that use no animals at all in any process of the work. They have been given to doctors in India, England, and Poland and a number of other countries. This has been promulgated by the IAAPEA (International Association Against Painful Experiments on Animals, England), which does not work negatively, but works positively towards the finding of further and further alternatives to animal research.

VAN HOOSIER: If I understood Mrs. Stevens' comments correctly, she made an analogy between the situation of some community pounds and prisons of years ago, and implied that the scientific community should accept the responsibility for this. In my experience the scientific communities had little contact with city or county pounds and the procedures that are followed there.

In regard to Dr. Hummer's comment, certainly one very valuable aspect of training veterinarians, particularly those that may be involved in experimental animal work, involves the postsurgical care of animals. The way they gain this training is by survival surgery, albeit single procedures on each animal. But to make this feasible in many colleges we do need dogs, and certainly the pound sources are one potential source. So, I see an inconsistency in the position of the Humane Society that says that they are for the use of animals in teaching, provided that the letter and the spirit of the Public Law is met, and yet restricting their position on the availability of dogs from pounds to nonsurvival procedures.

Certainly the essence of many of the things we are working for do involve the training of veterinary students in postsurgical care, and college should be a realistic training ground.

JORDAN: Survival surgery is not allowed in the veterinary schools in England.

The thing that surprised me this morning was the fact that researchers do not insure that their animals are kept under ideal conditions, because obviously if you want to do a proper experiment you must reduce the variables to an absolute minimum, and if you are going to keep your animals in conditions that are not ideal, then you are going to influence your results.

The question I wanted to ask—in England, all experimentation comes under the Cruelty to Animals Act, which will be 100 years old next year. Nevertheless, it is a reasonable act, although we would like to see it changed, and one of the conditions of that is that experiments are recorded. The total numbers of animals experimented on are about 5¼ million. Of these, 31 percent are of a medical nature, and the remainder are of a nonmedical nature.

Can you tell me what the proportion is in this country? I presume that we are talking about all experimentation, because it seems to me that an awful lot of experiments of a nonmedical nature should not be allowed. They seem to me experiments that are designed to protect the companies against later legislation, should any problems arise.

HUMMER: I think the National Society for Medical Research (NSMR) would have the best information; probably you should talk to the audience.

ROWSELL: I would like to take exception to one remark that Mrs. Stevens has made concerning our self-policing system. I appreciate very much that we can't extrapolate from a country of 22 million people a method to apply to a country of 250 million people. I do think that it is important to look at what a voluntary control program has done in Canada, particularly when Mrs. Stevens and Dr. Hummer have both said that the American legislation is not without fault and that it really looks at minimal requirements with respect to the care of experimental animals. Also, I think that Dr. Jordan mentioned that the British legislation has been in effect for almost 100 years. Mr. Richard Ryder, in his nefarious book, *Victims of Science*, has indicated that the British legislation is not all that it should be. The Littlewood Commission in 1965 reported on the British legislation and said at that particular time there should be certain recommendations followed. Now, we have had several different governments in Britain since 1965, none of which has seen fit to follow the Littlewood Commission reports.

Now to get on with the Canadian program. We have, at the national level, a Canadian Council on Animal Care, which is a voluntary control program. It is supported by the National Research Council and the Medical Research Council. Unique to our program is that we involved members of the humane movement, representatives from the Canadian Federation of Humane Societies.

When we carry out our assessment programs at all research institutes, teaching institutes in Canada and drug-testing institutes, we involve a member of the humane movement in the assessment program as a full member of the panel.

I know of no legislative program and certainly no program that would allow, except a voluntary control program, the involvement of the animal welfare movement as a full member, to go into the laboratories, "behind the closed doors" so to speak, to look at the scenes behind. I think that this is something worth recording; that a voluntary control program, unlike a legislative program, is not looking at minimal requirements. It is looking toward optimal requirements, and I think this is the important aspect.

I would just like to say one thing, which is that we do think our program is successful in Canada. With respect to the use of animals in surgical experiments, I think it is important to record here that the recent Swann Report on the veterinary profession in Great Britain suggests that because of the difficulties of obtaining clinical material, the profession—that is, the veterinary profession—should consider the advisability of allowing students to do a limited amount of survival surgery on normal animals and under strict supervision.

Present legislation prohibits this practice, and the report suggests that should present levels of student expertise be found to be curtailed by a change in legislation, the public attitude to permit survival surgery could be justified.

GEORGE J. RACE

Biological Variability: Precision in Biomedical Research*

INTRODUCTION

Animals are highly variable and difficult to measure. The purpose of this conference is to discuss the precision of measurement and possible alternate methods for use of whole animals. It has been stated that unless the scientist can quantitate a subject under study, he in fact knows nothing about it. Total quantitation should always be attempted. However, I submit that, in biological terms, we know a great deal about many subjects and that we can learn much more about them without total quantitation. Precision of measurement and total accuracy implies quantitation of all parameters with establishment of a mathematical balance between them. Required first are precise instruments for measurement, preparation of specimen for measurement, proper analytic procedure for measurement, and, finally, mathematical equilibration of all parameters. We know many parameters. If, on the one hand, great contributions have been made through studies of gene expression and protein synthesis in *Escherichia coli* bacteria, at one and the same time, great contributions have been made empirically through whole body studies. Notable examples of the latter include drugs developed through empiricism such as digitoxin (foxglove plant of old England), reserpine (from India) for mental disease and blood pressure control, and colchicine for gout.

Biology is not a uniform science. Everything is time-bound, space-

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bound, and the animal or plant currently being worked on is but a link in an evolutionary chain of changing forms, none of which has permanent validity (53). The following sections will serve to illustrate this great diversity with the attending difficulty of measurement.

SCIENTIFIC METHOD

The scientific method has been formalized as involving six successive operations (62, 76): (1) a problem is stated; (2) observations relevant to the problems are collected; (3) a hypothetical solution of the problem consistent with the observation is formulated; (4) predictions of other observable phenomena are deduced from the hypothesis; (5) occurrence or nonoccurrence of the predicted phenomenon is observed; and (6) the hypothesis is accepted, modified, or rejected in accordance with the degree of fulfillment of its predictions. As stated by Medawar (56), all advances of scientific understanding at every level begin with a speculative adventure, an imaginative preconception of what might be true. Developments in science have led to a flight from teleology and a progressive movement toward explanation in biology (42). This explanation has involved a unification and a multidisciplinary approach to many scientific problems.

The betterment of health must be sought with the least possible risk to patients. However, any new drug or form of treatment, even when proved effective in experimental animals, is still nonclinical until tried in humans. Restraints on experimentation are necessary but may be serious hindrances (13) and would certainly have impeded and delayed the invaluable contributions of Andreas Vesalius, William Harvey, Walter Reed, and countless others.

ANALYTIC APPROACH

Biologic considerations require dealing not only with methodological error but also with normal variation, multiple genetic and environmental influences, and intraindividual and interindividual differences still within the normal range (86). Biologic scatter can be reduced if systematic components of intraindividual variation are defined.

A consideration for the investigator is the relationship of age, sex, and general health status, when looking at the Gaussian statistics. Equations may be developed to express demographic or nondemographic contributions to the individual's blood chemistry values. A set of demographically corrected reference values can be derived and applied to a selected group of patients or experimental animals (25).

Quality control and precision begin with the frame of reference. In the clinical lab, this is often pooled serum (3). It is often improved by duplicate analyses, and two sample techniques serve for quality control. In biological research, such standards are not often available. We depend on pooled research results.

Biological observations are variables that may be of two types. They may be discontinuous with various intermittent discrete units measured, such as number of animal deaths in an experiment, or, secondly, they may be a continuous variable that can occur at any value with a graded logarithmic or other type of curve (65, 36). We are all familiar with standard tests, such as Student's "t" Test, Chi-square Test, and the Gaussian curve, but we are less familiar with the difference in the terminology of precision and accuracy (83). Accuracy may be defined as the mean measurement that is closest to the true value; that is, in fact, an absolute mathematical determination. Precision, however, represents that value obtained by repeated examinations of the same material, and the test of precision is the ability to reproduce the same number interchangeably and repeatedly over a period of time. Confidence limits expressed at 95 percent usually represent the two standard deviations or coefficients of variation limits that have become customarily associated with a 95 percent confidence level or a 5 percent error level (2 SD, $p = >0.05$).

Apart from biological variance, biological significance of a given level of enzyme or hormone, for example, depends not only on its quantity but also upon its physiological and biochemical method of attack. Small quantities of parathyroid hormone regulate large amounts of calcium.

One other term, the *reference sample*, should be mentioned. In the clinical lab, it may be pooled serum or a commercially purchased standard. In the investigator's laboratory, it will, by necessity, be a measurement against a previous known or calculated or presumed value, except for comparison with literature research results from many laboratories or devised negative and positive controls in the subject experimental animal group (37). For example, numerous factors can affect measurement of serum enzyme activities and contribute to variability of enzyme assays (30). Several clearly interdependent aspects are involved, such as serum-handling procedure; the effects of temperature on enzyme assays; the effects of different methodologies; the influence of magnitude of calculation factors; clinical instrumentation; and the interfering factors, such as absorbents, molar absorbance coefficient, reaction time in minutes, light path in centimeters, total assay of the volume, sample volume micromoles per milliliter per minute, calculation, wavelength accuracy, photometric accuracy, photometric linearity, and stray-light entrance, including the methods for assay (65).

Inherent in the concept of what is a normal range is the concept of a normal state. Normal has been taken to be ideal (5). Intraindividual variation, otherwise called physiologic variance in test values, has been found to be considerably narrower than interindividual variation or genetic variation. Between the extremes of intraindividual normal values and population normal values, there are many varieties of intermediate values.

Johann Karl F. Gauss's law of errors (17) was applied originally in the nineteenth century to repeated measurements of an attribute of a physical object; for example, the length of a stick. In the absence of any systematic bias, errors of measurement would be random and a frequency plot would distribute them in a nicely symmetrical bell-shaped curve around the mean value, which presumably is the true value. The measurements within populations of humans, even healthy adults, will also show a distribution pattern that is more often assumed than real.

Absolute research quality control may not always be a necessary factor in certain highly significant biological investigations (16). For example, the study of the morphology of delayed hypersensitivity in man with definitions and quantitations of types of cells, tissue pathology, electron microscopy, and a comprehensive group of findings leads ultimately to a concept of the process occurring. Total quantitation is not always possible (19). For example, quality control in laboratory investigation ensures similar results only to the degree that they can be measured. Cytological material examined in six centers by competent cytopathologists resulted in a degree of agreement that was acceptable with 3 percent false-negative and not more than 1.7 percent false-positive. Cytodiagnosis in itself is an expression of opinion. Scientific mensuration may be less than absolute.

BIOLOGICAL VARIATION

Linnaeus devised a system forming the basis for taxonomy of all animals today (39). It is clear that many inherited features of man or animals are the result of mutual actions of many genes. In addition to genetic control, all physical characteristics in a population have some undetermined limits of plasticity, depending on climate, environmental conditions, diet, and other changing factors (41). Also, biological plasticity occurs due to oxygen tension, altitude, radiation, stress, injury, and sickness. Indeed, one severe infection during the active growth phase of an animal may contribute to a 10 percent reduction in body size. The absence of such a "stunting" process has been proposed for explaining the increasing size of humans since the advent of the antibiotics.

An extreme adaptation is control of the blood pressure responses of wild giraffes. Due to the long neck of the giraffe, cerebral perfusion pressure with the head lowered may be 280 systolic and 180 diastolic millimeters of mercury, while with the animal erect they may be at 125/75 millimeters of mercury, a usual physiological range normal to other animals, including humans (82). The giraffe offers an ideal opportunity to study physiological control of such great variations in cerebral blood pressures through a series of check valves protecting cerebral capillaries, and thus the animal, against major cerebral hemorrhage. The literature is replete with examples of variability in biology, such as the transparent ice fish, which has no hemoglobin, or the hagfish, which has four hearts.

Comparative physiology among humans and air-breathing aquatic animals such as seals and whales is of importance. The respiratory adaptations in the diving seal are unique (67). The ability of the animal to cope with oxygen depletion means, in the final analysis, that there must be simultaneous adaptations at the cellular level to permit adequate generation of energy despite very low oxygen tensions in the circulating blood. The relation of oxygen depletion to continued cellular function is not well understood, but offers a potentially exploitable area of study in humans.

From the study of whole animals and organs, we have progressed more recently to the study of cells and organs in culture and cell organelles derived therefrom.

The research challenge is to adequately control the indeterminancy in biology (53) due to:

1. Randomness of an event with respect to its significance, i.e., mutations.
2. Uniqueness of all entities at the higher levels of biological integration, such as various unique life cycles of the mosquito, malarial parasite, dog, bird, and man.
3. Extreme complexity and infinite chemical parameters.
4. Emergence of new qualities at higher levels of integration, i.e., evolution.

All of these, individually and combined, reduce the precision of prediction in biological investigations.

THE WHOLE ANIMAL IN RESEARCH

In 1971 the National Academy of Sciences sponsored a symposium entitled "Defining the Laboratory Animal" (14). The conference topics relating to precision in laboratory animals are too numerous to discuss here. However, factors relative to precision in animal research included increased litter size in mice, brother–sister mating, cross-fostering, and sex standardization (unisex litters) (43). The well-defined mouse was affected by traffic in the cage room, number of people, number of mice in the cage, air temperature, air supply, food, bedding, water, type of cage, cleaning equipment and chemicals used for disinfectants, vermin, type of drinking bottles, humidity, light, noise, pheromones, and microbial environment—to name but a few variables (74). Also, inapparent laboratory infections represented a constant hazard to research quality control and standardization. The choice of a mouse or rat for research necessitates that factors be defined and controlled.

Wild Syrian hamsters, a common research animal, represent the descendants of two females and a male, all littermates, captured in Syria in the 1930's (6). Subsequent genetic subspecies show a high degree of histocompatibility through having common genes and, therefore, are potentially useful in transplantation research. However, mutations and inbreeding have now produced new strains of hamsters extremely susceptible to carcinogens, strains with lethal coat-color markers and hydrocephalus and others with unique pharmacogenetic traits (37a). Genetic history here is crucial to the research progress.

The rabbit is a well-defined laboratory animal (85) and has been used extensively, as have mice, rats, guinea pigs, and hamsters. However, the pig is more similar to humans and is an underutilized research animal (8). Its use as a laboratory animal is deterred perhaps by its large size, but a solution has been the development of a race of miniature pigs bred for laboratory use. It has been used extensively to study the effects of lethal radiation. Because of size, the pig has been particularly useful in operative technique development such as in cardiovascular surgery, open-heart surgery, and transplant surgery.

The use of rats, rabbits, and guinea pigs in biomedical studies is often better replaced by employment of nonhuman primate species because of their nearer phylogenetic relationship on the evolutionary scale, which makes significant results more applicable to man. Man's closest relatives, the chimpanzee *(Pan troglodytes)*, the gibbon *(Hylobates lar)*, the orangutan *(Pongo pygameas)*, and the gorilla *(Gorilla gorilla)* are followed by monkeys, baboons, and other primates. Indeed, primates represent the only species available for more advanced behavioral studies (28).

Protein comparisons allow establishment of commonality of polypeptide chains in various species, commonality of blood groups, and blood antigens that can be identified by specific antibodies such as anti-A, anti-B, anti-M, and anti-N. There are enough antigenic similarities between man and nonhuman primates to blunt the immune response to human HLA antigens. Thus, tumor membrane antigens from human melanoma can stimulate simian antihuman melanoma antibody for research and study of human tumor cells (73).

Primate research centers were started in 1960 on the premise that research into human disease requires a close relative in which the disease can be duplicated and studied, the cause and effect documented, and effective means of prevention and treatment developed (28). One area of research, for example, involves the anthropoid eye, which, with its stereoscopic vision, shows a portion of each visual field sent to each cortex through the mixing in the optic chiasma. Most other mammals fail to possess this stereoscopic arrangement and see only one side with each eye. In eye studies, primates are first choice for control similarity (39).

Atherosclerosis can be studied in monkeys with control from birth to death and dietary intervention to settle the question of atherosclerosis and fatty acids, cholesterol, diet, and their interrelationships.

The development of experimental surgical techniques in the pregnant rhesus monkey *(Macaca mulatta)* has resulted in major advances in intrauterine surgery for humans. Fetal immunological study and maternal antibodies are of immense importance. Transplantation studies between related primates have contributed greatly to the human renal transplant studies (from mother to son, and so forth). Primates offer the only promising studies for the slow-growing viruses that produce such diseases as kuru. Primates make a great contribution to the study of hazardous environments and long-term exposure to minimally toxic agents. Primates can be taught to smoke for toxicological investigations.

Primates have been essential in establishing fetal hormone effect on the differentiation of the central nervous system (66). The information is of extreme usefulness to the study of pregnancy and the development of the human fetus. Marihuana syndrome can be simulated in group-living macaque monkeys (70). Whether long-term frequent use of marihuana (tetrahydrocannabinol) can lead to persistent changes in behavior in neural endocrine function in man is still being debated.

Meanwhile, short-term and long-term behavioral effects of the drug in primates can be studied (35).

INBRED VERSUS HYBRID ANIMALS

The null hypothesis of R. A. Fisher (22) must be included here. Basically, the concept is one of likelihood of finding results of significance. The experimenter calculates deductively from the hypothesis the probable result of the experiment and compares it with a hypothetical null where the result is only due to random chance occurrence. A common fallacy consists of taking a single homogeneous sample. The proper procedure is to have several different homogeneous samples by using a plurality of pure lines or, preferably, F_1 crossbreeds to allow for analysis between samples. Without crossbreeds, the individual deprives himself of the possibility of making precise estimates for the error (40).

There is evidence that pure lines of mice are much less homogeneous genetically than is widely believed. It also appears that, in the case of some multifactorial characters at least, the homozygous state tends to be less stable developmentally than the heterozygous one. Grüneberg (29) states that:

> The use of inbred strains of mice and other rodents has been widely recommended for experimental purposes. The advantage of highly inbred over ordinary mice is held to be that the members of an inbred strain are genetically very similar and, hence, presumably more uniform both anatomically and physiologically.
>
> The confidence with which inbred strains have been recommended for general experimental use rests largely on the prestige of the mathematical theory of the results of inbreeding. . . .
>
> That pure lines of mice do, in fact, split up into genetically distinguishable sub-lines in the course of continued inbreeding has become obvious in recent years through the appearance of coat colour mutations, of histo-compatibility genes, etc. . . .
>
> While, in some of the cases . . . pure line animals are more variable than heterozygotes, the situation is clearly quite different, for example, in the case of antigenic characters (histo-compatibility genes, etc.) where, in tissue transplantation experiments, the reaction of pure-line animals is vastly more uniform than that of a genetically mixed batch of mice. Similarly, in cancer research, the superiority of pure-line mice has been established beyond doubt.

McLaren and Michie (55) state that:

> The evidence suggests that the widely adopted policy of using inbred strains for bio-assay may be a mistaken one, and that inter-strain F_1 hybrids are to be preferred. Such material combines the genetic uniformity of inbred strains with the "buffering" action against environmental variations which heterozygosity exerts.

Also, Festing (21) points out that inbred strains, F_1 hybrids, and noninbred strains should all be used in research. A theoretical number of F_1 hybrids between lines is very high. A single inbred strain of F_1 hybrid represents a single genotype. As the magnitude of genetic control increases, the gains in precision should increase.

If genetic factors are of minor importance, then it will not matter which strain is used; while if genetic factors are of major importance, noninbred strains would be inefficient and would not indicate that the character in question was strongly inherited. *The best experimental material is a mixture of genotypes arranged to be representative of several genotypes giving high-precision information on important genetic factors.*

Nevertheless, specific genetically controlled metabolic models may be bred and used especially in metabolic research. A model of proposed interrelationships among metabolic effects induced by lethal yellow A^y mutant in the house mouse (87) shows many features relating to protein metabolism, insulin secretion, obesity, hormone effects, hepatoma development, and so on.

CELL CULTURE

The usefulness of animal cell cultures as an experimental tool in such diverse fields as biochemistry, biophysics, genetics, virology, and cancer research has become increasingly evident in recent years (68). Standardized culture media is now available and remarkable metabolic tracer studies using ^{3}H-leucine and ^{14}C-thymidine for controlled study of the synthesis of nucleotides and protein complexes are available. Cloning can be studied. Genetic effects after exposure to radiation, chemical carcinogens, and oncogenic viruses can show an immediate or long-term effect. An example is mouse LS cells grown in steady-state suspension, which allow maintenance energy to be measured at 19 picomoles of ATP per cell per day, while it takes 22 picomoles of ATP for growth energy (77). The efficiency and energy requirements of protein synthesis can be studied.

Cell culture is employed for vaccine production; the cells used include rabbit and monkey kidney cells, chick and duck embryo fibroblasts, and diploid human fetal lung cells. Monkey kidney cells have been shown to harbor many viruses, including the simian viruses, SV1 to SV49. These adeno- and picornavirus groups may be oncogenic, especially SV40. Also, herpesvirus may be involved in the transformation of primary hamster kidney cells (38) in culture. Spontaneous neoplastic conversion of C3H mouse cells can take place in a serum-free chemically defined medium. Measurements and correlation of the factors of neoplastic transformation with the altered biosynthetic patterns are a potential source of very useful study (34).

Cell culture is especially useful in observing transformation in malignant development of cells under the influence of oncogenic viruses and chemical carcinogens. In the cell culture, alterations in the colony morphology and growth properties occur. In cancer tissues there is diminished adhesiveness of the cells, the adjacent cells, and a loss of surface substrate dependence (69). Cell culture allows investigation of the many primitive and differentiated cell types found in humans and animals. Cell culture offers the opportunity to manipulate single genes, single enzyme systems, single protein synthetic sequences, single nuclear derangements, and to study genetic engineering for correcting genetic defects.

GENETICS AND CELL HYBRIDS IN CULTURE

The ability of certain viruses to induce formation of multinucleate cells by fusing together cells of totally different animal cell types produces the artificial animal cell termed a heterokaryon (32,33). HeLa-type cells from human carcinoma may be combined with mouse Ehrlich ascites tumor cells. While they remain multinucleate, most heterokaryons do not multiply, but may survive for as long as 5 days. Failure to multiply appears to result from the cell having more than one nucleus. However, after fusion of the nucleus, mitosis will occur. These fused cells contain many nucleoli and may have a bizarre nuclear shape. Studies with tritiated thymidine can elucidate the chemical DNA synthesis in the nucleus. The hybrid cells synthesize protein and both sets of nuclei synthesize RNA. Autoradiographic and chemical techniques for the study of RNA metabolism in mammalian cells have reached a degree of sophistication that makes possible experiments never before accomplished.

The growth of mammalian cells from one mitosis to the next is characterized by a changing pattern of synthetic process (49). Hybrid cells may show enzyme markers available in somatic cells, because different species contain slightly different forms of the same enzyme (18).

The development of techniques for *in vitro* cultivation and fusion of somatic cells has provided a powerful impetus to study mammalian genetics (32, 52, 64). These techniques offer many options for chromosome study of highly complex mammalian cells and provide rapid progress in human chromosome mapping. Mapping may be determined by the existing hybrid cells through gene expression. The objective is to permit development of the human chromosome map comparable in detail to the one for the bacteria *E. coli* (72).

As scientists, we should note that the achievements in genetics in the past 50 years, in analyzing heredity variation and in explaining evolution, present a problem in principles for modern biology (12, 27). In evolution we see selected individuals and groups of selected individuals. In heterokaryons there is no hereditary long-term continuity. However, *in an outbred whole animal heterogeneous society, continuity is concealed and individuality is paramount. In an inbred homogeneous society, individuality is concealed and continuity is paramount.* We must select which we want.

Recently the development of animal chimeras with genes that have been sequestrated from different cell lines may offer additional opportunities for research (54). There may be complex interactions; chimeras are the product of more than one zygote. Fusion of embryos offers a chance to study genetic factors affecting many different areas. Study of polyclones has been extremely important in the identification of insect epithelial boundaries (11) as distinct from the progeny of chimeras (54). Chimeras that have different embryologic origins are genetically different tissues.

Consideration should be given to the serum protein polymorphism (78) as exemplified in the Atlantic salmon, in which the genetically isolated populations exist. Individual groups are sufficiently distinct for classification into a subspecies of the salmon group. For example, the frequency of the TF2 gene is highest in salmon population from the rivers in the southern part of the British Isles and lowest in the northern regions (61). Cell hybrids offer exceptional opportunity to study specific genetic markers, exhibited by cells of animal groups where measurable polymorphism exists.

ORGAN CULTURE

The controlled growth of an organ in whole or in part opens vast potential for research exploitation. Cardioactive drugs and the development of fetal hearts and their survival in organ culture make it possible to study synchronous beating and susceptibility of the muscle to toxicity of compounds at various stages of fetal development. Beta adrenergic blocking agents and catecholamines do have different effects in hearts before and after innervation of the heart. Mouse fetal heart is used, but presumably human fetal heart can be studied (1). Survival time, beating rates, cell membranes, and supporting oxygen and carbon dioxide can be studied in a special chamber with hyperbaric capability where one can carefully control Po_2 and Pco_2. The detrimental effects of hyperbaric oxygen can be demonstrated in the cultured organs (60). Also, organ culture can be done in the manner in which it can be observed microscopically. Tendons can be grown by a coverslip technique or a flask method and placed directly under the microscope (71).

For study of cell differentiation, the amphibian is ideal because it can regrow lost organs and appendages, and thus the study of tissue differentiation and regeneration is possible. *Morphogenesis* has been studied in avian and mammalian embryonic fetal tissues, as well as *morphostasis*—meaning the maintenance of normal differentiation and function of some adult mammalian organs, such as lung, prostate, skin, liver, kidney, pancreas, and spleen (59). Adult organ cultures are of great potential importance in seeking solutions to the entire problem related to carcinogenesis. Organ cultures can be used for detailed investigations of differences of cell size, DNA content, and protein synthesis (50). Study of the cell cycle in specific inhibition or stimulation can be made. Study of visceral organs of adults and control of major metabolic processes and action of hormones, such as adrenal and thyroid hormones, and the effect on protein synthesis are very productive. For example, embryonic thyroid glands cultured for 4 days to study the effect of iodine metabolism will show the ultrastructure of the rat thyroid to be maintained in an essentially normal condition. Some of the histological changes that do occur are a direct result of the cessation of synthetic activity by the follicle cells and the resorption of colloid from follicles (2).

Differentiation of taste buds, innervation of the iris, retinal embryogenesis and hemangioblastoma have been organ cultured with great effectiveness (20,75,79). By growing the epithelial surface of tongue in organ culture, histologically the papilla can be seen covered with layers of epithelial cells, but no taste buds are present initially (20). Explants grow well for up to 15 days, and taste bud structures will develop. Development of neurons can be seen during innervation of the taste buds. This system is devised to investigate the formation of the neuromuscular junctions *in vitro* and permits study of fundamental problems concerning growth induction and cell recognition during development.

Organ culture is particularly useful in studying reinnervation of tissues by nerves (75). For example, in artificial medium the sympathetic nerve will reinnervate the rat iris of the eye when in contact with a superior cervical ganglion in organ culture. This will occur even across species where the rat iris of the eye is in contact with a superior cervical ganglion in organ culture. The rat iris can be photographed at multiple stages, and the ingrowth process depends on nerve growth factor, a protein that is distributed among many species of animals, including man. Very little is known about the necessity of and the factors responsible for reinnervation of peripheral tissues or synapse formation in the central nervous system. Organ culture systems are useful tools in research and control of nerve fiber growth.

In summary, organ culture respresents a situation whereby physiological systems and biological processes may be manipulated for integrated study as opposed to a single biochemical study or observation. Independent differentiated cell lines may fuse to produce a designed organ capable of function, e.g., an innervated beating heart.

COMPUTER-ASSISTED RESEARCH

The computer offers untapped and near limitless innovations for research. It can be used for statistical calculations, factor analysis, probability factors, matrix manipulation, axis rotation, factor norming, searching for missing parameters, and predicting the same. It can be a tool of labor for counting cells, sizing cells, sorting cells by electrical charge, fluorescein dye staining, and so on. An image analyzer may scan cytologic smears for abnormal size, chromatin content, and quantitation of DNA and suggest malignant characteristics. For example, eight response variables indicative of cell growth can be measured, including area of the cell, area of the nucleus, area of cytoplasm, length of the cell, lengths of the major and minor axes, number of nucleoli per cell, and total area of nucleoli per cell (84).

An automated image analyzer has been used to count the lactate dehydrogenase-positive alveolar wall cells in the lung (type 2 pneumocytes) in guinea pigs injured by nitrogen dioxide (51). These were counted on photographic slides in microscopic fields; a high coefficient of correlation was obtained, and time required for counting was reduced from several weeks to less than an hour, with greater precision and accuracy in the automated counts.

In renal physiological simulation, a mathematical model of the renal medulla can be made, integrating structural and functional concepts into a unified understanding of the mechanisms involved. Numerical studies can be carried out to simulate the movement of water, sodium, and urea in nonsteady states on an assumption that sodium pump exists in the kidney. The model yields piecewise continuous curves for volume flow rate and sodium and urea concentration profiles that reasonably agree with available data (24). Computer simulation of osmotic gradient without active transport in renal inner medulla was studied by alternate models of passive and active water reabsorption in Henle's loop. Computer simulation of the model could show an osmotic gradient in the inner medulla, on condition that sodium movement out of the thin ascending limb resulted in the tubular fluid becoming hyposmotic to adjacent interstitium. The importance of urea was illustrated in the model, first by simulation of a low-protein diet and secondly by simulation of water diuresis. In both situations, reduced reabsorption of urea from the late collecting ducts caused a reduced interstitial urea concentration, which in turn caused a severe reduction in interstitial electrolyte concentration (80).

In cell kinetics, computers are used to analyze variations in the experimental asites tumor (L1210 murine leukemia). The cell populations were designated as models I, II, and III. The proposed three alternative hypotheses in cell kinetics of the ascites tumor are fitted to each model. The observed data growth curve percentage of labeled mitoses was analyzed on the computer based on alternative mathematical models for cell kinetics (15), and the most probable process was identified.

For the clinician, the computer can be used for clinical diagnosis, personality assessment, and teaching; for example, as an integral part of instruction in the normal and abnormal cardiac cycle. The simulations are developed by analog–logic computer and include the heart sounds (visually and audibly); the ECG; ventricular, atrial, and aortic pressure; aortic flow; and ventricular volume (57).

In summary, where hard numerical data exist through study of animals or cells, the computer offers an unparalleled opportunity to predict and quantitate the missing parameters and permute trial hypotheses until a likely answer is found.

PHARMACOGENETICS

Hereditary variance in drug response is a major new area of pharmacology. Medicinal plants were well established in the day of Columbus, who had his plant book with him on his first trip.

Great contributions have occurred without great knowledge of pharmacogenetics. For example, Dr. Philip Hench gave us cortisone for arthritis. He had observed remissions of rheumatoid arthritis in pregnant women. He gave his nonpregnant hospitalized patient an initial injection of compound E.* On the third day she was out of bed and on the fourth day went on a shopping tour. Obviously, cortisone had a profound biological effect and opened an entire new series of science and medicine in the adrenal cortex function. All these observations were empirical demonstrations of the biochemical effects of the adrenal glucocorticoids.

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*Cortisone (17-hydroxy-11-dehydrocorticosterone).

Pharmacogenetics has disclosed some heretofore unsuspected genetic differences among men (26, 48). In practical terms, pharmacogenetics should aid the physician in choosing the right drug in the proper dosage for each individual. Also, it should help to define more sharply the limits of safety and effectiveness of many drugs, both new and old.

From their work on mutations produced in a mold by X rays, George W. Beadle and Edward L. Tatum, Nobel Prize winners in 1958, concluded that each gene may be responsible for the specific nature of a single enzyme. A mammalian cell may have 3 million genes. When the gene is functioning normally, the enzyme it controls operates efficiently. If the gene is damaged, the activity of the enzyme will be impaired or destroyed. An example is hemoglobin Zurich, which has the amino acid arginine in place of histidine, thus rendering the red blood cells susceptible to hemolysis from sulfonamides (23). Heritable drug responses can be compared in pairs of identical (homozygous) and of fraternal (heterozygous) twins. Ideally, the two members of a pair should live apart, so that environmental factors will be equally diverse for all subjects and genetic factors will dominate. The formula for drug genetic influence is:

$$(\text{Variance within pairs of fraternal twins}) - (\text{Variance within pairs of identical twins}) \div (\text{Variance within pairs of fraternal twins})$$

Computation gives values from 0.01, indicating a negligible hereditary contribution, to 0.99, indicating virtually complete hereditary influence. For some drugs, ethanol included, the values exceeded 0.97. Separately considered is variation in bioavailability, which is not a new phenomenon. Sixteen different tetracycline-containing preparations were studied, and seven were shown to provide plasma levels less than the minimum concentration (48). The apparent difference is a pharmacokinetic problem.

Mechanisms and examples of drug idiosyncrasies include drug toxicity due to autosomal deficiency of cholinesterase enzyme, increased sensitivity to nitrites due to the presence of abnormal hemoglobins M and H, and novel unexpected drug effects such as favism or hemolytic anemia induced by primaquine in the hereditary absence of glucose-6-

phosphate dehydrogenase in erythrocytes. Drugs may have decreased responsiveness due to the absence of a necessary cofactor, such as vitamin B_{12}, which is unresponsive in the absence of the intrinsic factor.

Persistence of methadone-^{3}H and its metabolites in rat brain after a single injection has its implications of pharmacological tolerance (58). Genetic control of barbiturate depression can be demonstrated in paired genotypes obtained by selective breeding and designated as BSVS (sensitive) and BRVR (resistant) with respect to certain bacterial and other infections (31). These animals, bred to be tolerant to or resistant to certain infections, exhibit a similar sensitivity and resistance to pentobarbitone (phenobarbital) and hexobarbitone (hexobarbital) as established by dose-response curves. More animal models with specific hereditary defects of drug metabolism need to be bred and maintained for drug testing.

Pharmacogenetics and pharmacokinetics offer predictable explanations for bizarre drug reactions. Pharmacogenetic studies should be expanded not only for the protection of individuals against adverse drug reactions, but also because such studies may reveal unsuspected relationships between unusual drug reactions and susceptibility to diseases of various kinds.

BIOLOGICAL VARIABILITY IN IMMUNE RESPONSE TO ANIMAL PARASITES

The role of immunity in parasitism is a special immunity. Survival of the parasite species depends upon its adaptation to the host. Protozoa may, for example, continue to multiply and overwhelm their host. Trypanosomes (10) may live in near harmony with their host for long periods of time. Predators kill organisms that they prey on, but parasitic life forms establish an order for a new life cycle. The parasites remain alive as long as their hosts remain alive. Two specific immunosuppressive systems are operative (81).

Parasites may enter macrophages and survive by evading the interaction between sensitized T-lymphocyte and antigen, by preventing fusion of lysosomes and phagosomes, or by neutralizing lysosomal enzymes. Parasites may survive by antigenic variation, by immunosuppression of the host immune system, by uptake of host antigens (wolf in sheep's clothing), by producing soluble blocking antibodies, by neutralizing activated macrophage response, and by encystment where the cyst is effectively isolating the parasite from the host.

Parasitic immune responses are often indecisive as compared with yes–no total responses typical of bacterial immunity. However, tubercle bacilli in macrophages behave as parasites often do. In parasitic diseases, many antibodies can be demonstrated. For example, complement fixation, agglutination, fluorescent antibody, immunodiffusion, diatest, circumoral precipitation, delayed hypersensitivity, and immediate hypersensitivity are characteristic in variable degrees of protozoal diseases such as amebiasis, malaria, leishmaniasis, trypanosomiasis, toxoplasmosis, trichinosis, filariasis, schistosomiasis, and echinococcosis (10).

Antibodies can be passively transferred; however, circulating antibodies are generally not very protective. In some instances a parasiticidal antibody has been revealed by *in vivo* assays. In this case, the parasites are actually killed and eliminated, essentially before the disease develops.

An example of the difficulty of study is in malarial infections. Brown (7) states there is antigenic variation to malaria experimentally proven in *Plasmodium berghei* in mice and *P. knowlesi* in monkeys. Two levels of antigenic diversity are known in erythrocytic plasmodial infections. The onset of clinical immunity coincides with the T-lymphocytes' activity, which plays a crucial part in the response. Their role appears to be that of helper in antibody synthesis, which also includes B-lymphocytes. The onset of protective immunity is characterized by a sudden drop in the number of parasites present in the patient's blood in either subclinical or clinical disease.

An appreciation of antigenic differences and strains and antigenic variations is absolutely essential to understanding the immunology of malaria. They are an integral part of the shifting balance of host–parasite relationship. Antigenic variation in African trypanosomes shows that parasites seem to have the ability to change the surface antigens. The host then is confused by the disguise, which appears to recognize the surface of the invader as a "self-material" when it is, in fact, a host protein coating of an invading parasite. The researcher must have a control for each variant.

Beale (4) suggests that paramecium cells are able to switch from one state to another, each state being associated with a particular expression of a gene and antigen, enabling the parasite to evade immunological reaction of the hosts. Antigenic variation is obviously a most important protective mechanism to the parasite, allowing it to survive.

Clegg *et al.* (9) show that evasion of the immune response by the adult schistosoma may be due to the hostlike antigen terminals on the surface of the membrane that block the attachment of antibodies

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against the parasite. Antigenic blood determinants may be acquired by the schistosomes cultured in human blood. Research control is difficult.

Trichinella spiralis, 1954–1975

In my own work with John Larsh of the University of North Carolina, we have attempted to standardize immunological reaction in mice infected with *Trichinella spiralis* (47). The strain of *T. spiralis* that we have used was isolated from a pig in 1936, maintained in laboratory rats for 7 years, and then maintained thereafter in our strain of randomly inbred (35 years) Swiss Webster mice (by Dr. Larsh).

Initially the mice were sensitized by three separately spaced stimulating infections of *T. spiralis* (44). Most worms were localized in the anterior half of the small intestine. The sensitized mice expelled a significant number of worms from the entire small intestine between 5 and 7 days, whereas in the nonsensitized controls this period of time was extended between 11 and 14 days.

Histopathologic study with sensitized and nonsensitized mice was reported in 1954 (45). No evidence of an inflammatory response was noted in the anterior half of the small intestine of the nonsensitized mice until 4 days after the challenging infection. The zenith of acute inflammation occurred on day eight, followed by a chronic, diminishing phase. Histopathologic evaluation showed inflammation already had been initiated in the tissues of the sensitized mice by 12 hours after this infection. The peak of the acute response in immune mice was reached by 4 days, compared to 8 days in nonimmune controls. After the peak of the acute inflammatory response in the anterior half of the small intestine of both groups of mice, there was a significant loss of adult worms from the entire small intestine. This close temporal association suggested that inflammation was the *direct* cause of the expulsion of the worms (47).

These studies raised the question of what is the cause of the injury that triggers the inflammation? The answer to this question required a determination of whether the immunity demonstrated against the adult worms was humoral or cell-mediated.

Because lymphoid cells transferred from sensitized donors produced adoptive immunity in recipients (46,47,63), we suggested that delayed hypersensitivity (DH) is causally related to the expulsion of *T. spiralis*.

Based on many protocols and studies over 20 years from our laboratory with the same strain of parasite and host, it was apparent that (1) there was a close, direct association of a characteristic pattern of acute intestinal inflammation and subsequent expulsion of adult worms of *T. spiralis* and (2) there was a similar association between the degree of sensitivity of the host at challenge and the timing and intensity of inflammation and loss of worms from the intestine. The results in all instances were considered to be consistent with this hypothesis of hypersensitivity. Finally, the results obtained in prior studies in the 1950's are easily reproduced today in this model of parasite and host.

We may say that the future of research has never been more potentially fruitful. Nevertheless, I quote the "Harvard Law of Animal Behavior," of which I have heard through tradition and not through publication, which states: "Animals under the most precisely controlled laboratory conditions still do as they . . . please."

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DISCUSSION

PRATT: I was struck by the many things that Dr. Race mentioned in discussing variability in experimentation, but I was also struck by one that he omitted. He didn't mention the effect of pain on the experimental results, although he mentioned such factors as litter mates, temperature, and many others in the environment.

I am not only speaking about the so-called unnecessary pain, which was referred to this morning by Dr. Hummer as being a very bad thing that is taken care of, supposedly, by things such as the Animal Welfare Act. I am also referring to necessary pain, which is not eliminated by legislation, but which occurs in animal experimentation and is allowed to occur because to relieve it would *frustrate the object of the experiment*. That may be, but perhaps also necessary pain may have a considerable effect on the variation in the experiment.

I would also like to mention such things as anxiety, again, something that is rarely mentioned in published results of experimentation, but that I would imagine might also have an effect on the results.

RACE: There can be no doubt that anxiety has a very strong, sympathetic, hormonal, neurohormonal response, as does pain, and affects physiological function—heart rate, blood pressure, and so on. It definitely is a variable factor that, if included, probably diminishes the degree of precision and accuracy in an experiment.

THOMSEN: I would like to say first that quite a few of the humanitarians here have come a long way to get information. We would certainly appreciate it if the various speakers on the various subjects would address themselves not only to what is being done now, but deficiencies in what is being done now; for example, in the use of tissue cultures and so on, and possible extension of methods that would save animal suffering.

I would like to ask Dr. Race if he would venture an opinion, not in terms of 58 percent or anything of that sort, but from your observation and reading of the literature and so on, what general proportion of the experiments involving animals would you say have been done with reasonably good experimental design?

I might say that a study, made some time ago and undoubtedly a competent analysis, took a random sample of experiments involving animals and found that perhaps 5 percent of the experiments were executed and reported in a manner that would indicate adequate attention to experimental design and analysis of the results. Do you think that they are now observing proper experimental design in the great majority of experiments, or in a small minority?

RACE: I must say that proper designs are usually there. Each institution has a research committee and often a human-investigation committee and an animal-care committee. Now, most research protocols will be looked at by those committees, as well as by their department chairman. If the department chairman and committees find them inadequate, there are three or four good opportunities for correction of the design. I think most of them are well designed, or else they are heavily criticized before they are started.

THOMSEN: There has been a vast improvement, then, over the last 10 years.

RACE: Since the time I began in the early fifties until now, it certainly has improved.

SPIRA: Harlow, who was the editor for 12 years of the *Journal of Comparative and Physiological Psychology*, estimated that he reviewed 2,500 manuscripts. His parting words as an editor were: "Most experiments are not worth doing, and the data obtained are not worth publishing." And Harlow had a good bit of experience with experiments.

One of the things that bothered me today was that nobody seems to address themselves to the pain and suffering of the animal.

HARRELL: There will be papers this afternoon touching on these points. As you indicated, Dr. Harlow was speaking about experiments in the field of psychology, which is his own particular interest.

RACE: There is no question that every effort should be made to prevent animal suffering. I think that is an ethical moral obligation. There is also no question that every drug is nonclinical and nonproven, no matter how well it reacts in tissue culture to kill cancer cells, until it has subsequently been used in a whole animal that has cancer and subsequently in a human being who has cancer. At some point it passes the borderline from being something that is strictly investigational into something that can be useful potentially clinically. Of course, what we are doing is trying to strike a balance between all of the multiple needs and factors that I think we all have obligations to promote.

KURT BENIRSCHKE

Experimental Systems: Advantages and Disadvantages

We are examining the past, present, and future contributions of animals to human health and welfare and also whether alternative methods, such as cell or organ cultures, can partially or completely substitute for the intact animal model. In part, it would appear, the reason for this examination of our *modus operandi* is that the latter methods have become more powerful, more widespread, and also less expensive. At the same time, animal availability has become of concern, particularly that of nonhuman primates. This is attested to by two recent full-scale studies (12, 21). Other reasons for possible changes in attitude are discussed in other chapters in this volume.

I will address four difficult questions and provide some possible answers in the short space allotted. Broadly speaking, these questions are:

1. Are there valid reasons why certain experimentation requires intact animals, specific species, or developmental stages?
2. Can we depend upon valid experiments of nature (the so-called animal models for human disease), and how can they be recognized expeditiously and be usefully employed?
3. Are there specific limitations to their efficient use by virtue of lack of standardization or by nutritional or parasitic interference or the like?
4. When alternate systems exist (e.g., culture methods) for the study of diseases, do these have an impact on data that could be extrapolated to intact animals?

These are searching questions and only a superficial inquiry can be made of the vast body of literature that exists to answer each question. In order to avoid the cataloging of such relevant items, it will be my purpose here to highlight in each area certain contemporaneous problems that illustrate the point well. Moreover, because of my own background and interests, much of the discussion will be concerned with reproductive pathophysiology, not that other areas of disease would not equally well have made the point.

Are there valid reasons why certain experimentation requires intact animals, specific species, or developmental stages?

The answer must be an unequivocal yes to this question if it is our intent to further improve human health and welfare. Such a statement is perhaps most readily defended from the vista of microbiology. Were it not for the painstaking experimentation with animals that has ultimately led to our capacity to vaccinate against smallpox, poliomyelitis, and other virus-induced diseases, freedom from these scourges would hardly have been achieved. We accept this fact and many others of preventive medicine as "so-whats" today, and rarely do we examine the agonizing research that went into its development. This research also had many ramifications, not necessarily restricted to the field of virology. It first really opened the field of tissue culture, as well as that of medical primatology, to name two items that we also now take for granted.

I want now to illustrate three areas of contemporaneous research on intact animals whose execution in alternate systems cannot presently be envisaged.

1. *Leprosy*. It is estimated that 10.8 million people suffer from this mutilating disease and, lacking the availability of an animal model or successful *in vitro* manipulation of the organism, little hope for eradication of it has existed (10). Attempts to propagate the organisms in many species have had very limited results. Typical lepromatous lesions were not produced. Thus, research into the biology of the bacillus, effective drug therapy, even supply of lepromin for testing were severely limited. In 1971 Storrs (28) reported her success in transmitting the disease to the nine-banded armadillo. This species is remarkably susceptible, produces widespread lesions, and for the first time a rational approach toward the conquest of the disease has become feasible (17). It has been learned that a closely related species, the South American seven-banded armadillo, is also susceptible to the organism, and, most recently, armadillo cell lines have been produced and *in vitro* infection may become feasible soon (1).

At this time it is difficult to see how leprosy research can progress satisfactorily without this new animal model. One can anticipate that thorough study will not only elucidate the reasons why man and these two edentates are capable of developing leprosy, but also that more effective therapy, perhaps preventive vaccination, can eradicate the disease or at least restrict its occurrence substantially.

No restrictions to the usage of this interesting and beneficial animal have yet been implored, perhaps because by many it is regarded as a pest, and supply would appear to be adequate. Yet, there are many of the 20 species of armadillos, living only in South America, threatened with extinction, and importation of most is very difficult. Moreover, if this animal is to continue to serve as a singular species for leprosy research, and it might be added for research into the causes of monozygotic twinning, then husbandry has to be developed that enables ready propagation. Only rarely have these species been bred in captivity, and the basic reproductive biology is not understood to the point that colonies can be created, say in Africa, to get on with this research. For the time being, only culling from the presently still abundant wild population fills the laboratory need. From an ethical point of view it must be warned that feral armadillos have overrun Florida, a fate that could come to another country without sufficient safeguards.

2. *Chimerism*. This complex current topic is here introduced because I can see no possible way that animal experimentation can be avoided if an answer to one of the more important questions about a fundamental aspect of embryonic development, that of sex differentiation, is to be found.

When fraternal twins develop blood vessel connections in their placentas, immature blood cells exchange between them and induce in each other clones of foreign cells that render them "chimeric." Thus, a boy may have bone marrow elements from his fraternal twin sister and vice versa. Because of this embryonic exposure, they live in harmony with this foreign clone and do not reject it, as they would if the foreign cells had first been applied after birth.

The existence and recognition of this phenomenon in cattle frequently, and some other artiodactyls less often, has been the main impetus for the development of the advanced state of transplantation biology as we now know it. It was possible to recognize this "blood chimerism" because the freemartin condition in cattle had been well known to farmers for centuries but needed scientific explanation. The freemartin is a female, co-twin to a male, whose sexual development was thwarted, presumably by exposure to some endocrine substances issuing from the testis of her co-twin and transfused through the placental connections. She is barren, and economic considerations have thus caused many to look askance at the trait for twinning in cattle. Although recognized for over 60 years, the mechanism by which this sterilization of the female fetus comes about is still unknown (3).

A discussion of this embryologic phenomenon may seem redundant were it not designed to point out the need for experimentation with specific species. Blood chimerism has been recognized in occasional human twins, but the genital system of the female fetus is unaffected. It also turns out that all of the South American primates of the family Callithrichidae, the marmosets, always have fraternal twins and that the females of the 50 percent heterosexual twins produced are unaffected by the chimeric blood that is being exchanged through the placenta.

Experimental data have been produced that would exclude gonadal steroids as the causative agents in the sterilizing effect (15). These studies, performed on specifically timed embryonic rodent genital ducts explanted in organ culture, suggest the existence of a high molecular testicular agent capable of suppressing uterus formation in the female embryo whose nature is yet obscure. The salient question is why it should act in cattle but not under seemingly similar conditions in primates. Unquestionably, intact marmoset pregnancies will ultimately be necessary to provide the answer. And it would appear to be an important problem of comparative research.

We have reason to believe that embryological principles do not differ materially among mammals, only some details do. Nevertheless, these details are of great importance to understand, so as to enable us to make use of pregnant rodents and the like for the safety-testing of new drugs. Since it it unlikely that consumption and development of new agents will decrease in the future, appropriate safeguards are imposed by regulating agencies that prevent potential harm to the consumer. This includes the fetus, and I need only to mention thalidomide or stilbestrol in order to emphasize the need for valid experimental subjects during pregnancy.

The physiology of animals, particularly placental transfer mechanics, embryonic handling of drugs, length of gestation, and so on, are sufficiently varied that increasingly caution has been expressed at extrapolation amongst results from experiments in widely different taxa. Teratogenesis is of particular concern, and unforeseen things happen. At present, there is some preoccupation with disturbances of the sexual system because of the stilbestrol-related vaginal adenosis and adenocarcinoma. The recognition of this effect, incidentally, was predated by disregarded postnatal experiments in mice (11). Moreover, we share concern over the potential effects upon embryonic development of antifertility agents (8).

These considerations surely suggest that basic research in intact animals will be needed in this area and that safety-testing of drugs will have to continue in pregnant species. From what we now know, this will often have to be a pregnant nonhuman primate, although substitution with other systems has been pleaded for whenever possible (4).

There are currently enormous limitations in the supply of primates, and the, I believe justified, pressure to become independent of importation by creating adequate breeding facilities is finally being heeded (12). Nevertheless, greater concern for primates, because of their nature, will always exist than for "lower" species.

One obvious way to answer, in part, the important questions of human embryology that concern the pharmacologist and virologist, to name only two disciplines, is the appropriate usage of human tissues from abortions. It is at least as difficult to justify the rape of primates from nature for said purposes as is the measurement of drugs in intentionally aborted embryos or the determination of their effect upon development by dissection. Some relevant problems cannot otherwise be solved with current knowledge. For instance, the common cytomegalovirus infection of embryos, whose agent only propagates in human fetal fibroblast lines, will have to be investigated using human embryonic material.

Parenthetically, it might be mentioned that the more complex type of chimerism, that of the whole body, has been elegantly explored in animals by Tarkowski (29) and Mintz (20). It could *only* have been done in the intact animal, in this instance the mouse. The results have provided enormous insights into basic biologic phenomena whose answers are fundamental to our knowledge of embryogenesis and teratology.

3. A final reason why only certain intact animals at specific developmental stages are useful for experimentation with the ultimate goal of improving human health comes from the area of *inborn errors of development*.

Individually, metabolic defects are rare though cumulative, and, because they are hereditary, they are an important cause of human illness. Much progress has been made in the past decade in the prenatal diagnosis of genetic disease, but its effective therapy has been difficult. The diagnosis of some errors is hampered because of the need for fetal blood (22). That effective therapy of fetal diseases can be efficacious is attested to by the intrauterine transfusion of blood to erythroblastotic fetuses. In other disorders it would appear that enzyme substitution from early ages of embryonic development would be necessary, yet this has not been successfully accomplished so far. The first really important breakthrough has just been reported by Ampola *et al.* (2) in a case of fetal methylmalonic acidemia. Nyhan (23) comments editorially on the importance of this event and cautiously forecasts that other afflictions may be accessible to therapy in the future, pending better understanding of the pathogenesis and aspects of embryonic development.

Many animal models of human inborn errors exist whose *in vivo* study is widely undertaken. In particular, mice, but also cattle, minks, and dogs, to name a few, have identical genetic errors in common with man. Taking advantage of sophisticated *in vitro* cell culture and enzymatic assay systems, we are now coming to the era when enzyme therapy and genetic reconstitution can be envisaged in animals. For instance, the intentional induction of red-cell chimerism with normal hemoglobin manufacture could make infants, now doomed because of Bart's hemoglobinopathy (alpha-thalassemia), potentially survive. While the stage needs to be prepared with *in vitro* studies, mostly already accomplished, intact animal pregnancies will be needed before successful therapy in man can be practiced.

A prerequisite for such therapy ultimately is operative access to the fetus, particulary to the placental vasculature. Not only would this be required for blood sampling to allow diagnosis (already accomplished in early trials) (13), but also for the administration of cells or enzymes. Fetoscopy, first practiced in animals, and blood aspiration could be practiced only in sufficiently large animals. Ideally it is undertaken in a species with similar uterine and placental configuration to that of man. Here again, *in utero* experimentation with primate pregnancies paved the way to show that the fetus could be approached with relative impunity. Restrictions in human fetal research though, imposed in the past year, have forestalled a possibly more rapid development. The cost of pregnant primates, as well as their dwindling supply, make this a problematic, albeit necessary, area of future investigation for which no substitute can be envisaged.

One important area of concern is the development of graft versus host disease. With transfusion of cells as here forecast, the possibility of these competent cells rejecting the recipient with ensuing "runt disease" is not only a possibility, but also has been witnessed on occasion in the treated erythroblastotic infant. Irradiation of cells, antigen matching, and so forth, have all been practiced but are at best secondary means. More crucial for success is the delineation of the time sequence when chimerism can, and then can no longer, be induced so that all therapy can be administered by fetoscope at a prior date. Fanciful as these thoughts may appear, these modes most surely will be applied, and they cannot be done without experimentation in intact animals of specific types and only at very specific times of their development.

Can experiments of nature be depended upon, and how can we recognize and follow through on them?

Experiments of nature in this context are construed to be visible models for existing human conditions such as the chimerism alluded to before. They are not construed to be the availability of animals in which to create diseases similar to those in man, as leprosy in the armadillo.

In general, existing animal models simulating or exactly paraphrasing human disease are widespread, and they are commonly employed by investigators. So desirable are such models that a handbook for their description is under continuous publication, and National Research Council as well as Armed Forces Institute of Pathology committees endeavor to make these models more widely known and accessible (14). The reason for these activities is the desire to eradicate more quickly the numerous conditions in man whose conquest, we feel, should be at hand once the basic pathophysiology of the disease is fully understood. And precedent for this assumption, often based on the similarity of mammalian genomes and its relative conservatism during evolution, could be cited. Thus, X-linked disorders in man (hemophilia, G-6-PD deficiency) find their equivalent in many other mammals (24). So strong is our belief that the experiment of nature proves useful in human-disease studies that deliberate searches for models of such a common disease as cystic fibrosis have been issued (25). So far, a useful model has been elusive, or we may not have been wise enough to recognize it or exploit it if indeed such a model already exists. Similar laments could be made for models of "sudden death in infancy," which poses an important current challenge to society, or the widespread disease of pregnancy, preeclampsia.

Once a disease model is identified, can it be relied upon? The elucidation of chimerism earlier reported has given major new insight into the question of tolerance and sex differentiation. At the same time, it must be admitted that extrapolation to other species, including man, poses problems whose individual elucidation requires additional research. Witness that "tolerance of antigens" can be postnatally induced in mice immediately. Not so in cattle, man, or marmoset, in which the switch to immunologic competence proceeds before birth and at specific and individually different time periods (27). Nevertheless, the broad principle holds, and this is the promise of "experiments of nature." This is why pursuit of the question of sex differentiation may be usefully attacked with these models.

I would like to explore one more entity of an experiment of nature, parturition, because it simultaneously illustrates the complexity of research, the reasons for delays in recognition, and possible problems encountered in the pursuit of what starts out to be a superior model approach.

The time to be born is quite rigidly observed, and it differs from species to species. Thus, the mouse delivers her young in 20 days, the sheep in 150 days, the human in 270 days, and so on. Deviations from this regularity of birth are considered abnormal and often have grave consequences for the young and, in the case of postmaturity, for young and mother as well. Many speculations have been advanced as to the internal clock that regulates this ubiquitous event, but only very recently has the first insight come into this mechanism. It came from the recognition of experiments of nature.

Prolongation of pregnancy with neonatal wastage has been recognized in cattle for over 20 years. The mode of inheritance was delineated and elucidation of the pathologic findings in the neonates suggested as common feature a disturbance of pituitary/adrenal mechanics with different input in different strains of cattle. Next it was recognized that sheep terata, induced by accidental ingestion of various plants, also were carried past term and that they had similar endocrine abnormalities. These early observations set the stage for elegant hypotheses, but also for the experimental approach that was needed to verify the ideas and to define precise pathophysiologic events. Early results and the historical events are well delineated by Kennedy *et al.* (16), but the enormous complexity that was discovered subsequently could not possibly have been envisaged. It awaited simultaneous discovery of prostaglandins, as well as much additional knowledge of fetal endocrinology.

Liggins (18) has recently reviewed the work that now clarifies the central role of the fetal endocrine system in the initiation of birth in sheep, and it is apparent that none of these discoveries could have been made without experimentation in intact animals. At the same time, direct translation of this knowledge to the human condition is not possible. Likewise, differences exist between sheep and the birth of nonhuman primates. It would appear that additional primate investigations are needed before the principles find application to the human condition. Nevertheless, work done so far indicates that similar principles apply in man, although the details differ in many respects. From these studies, vast benefits accrue to human health already, and others can be anticipated in the future. Thus, the influence of adrenal hormones on the maturation of lung development has been recognized, and therapy of this nature is being applied. Also, experimental studies suggest that premature labor can be forestalled at times; a new enzymatic placental defect has been uncovered as a side finding.

Why did it take seemingly so long to come only this far? In part it was the need to commence experimentation once a hypothetical model was recognized. This is not everybody's forté, and motivated people had to come along. In part though, it necessitated the evolution of ancillary knowledge, in this case endocrinology and prostaglandin research. Their need cannot necessarily be anticipated nor the competence be created by fiat.

Doubtless, numerous animal models exist that are not exploited. The monozygotic littering of armadillos comes to mind, recognized in 1913 by Patterson. It is not being exploited, perhaps because scientists feel no need to bother with it or, in this case, because breeding of these animals has been difficult. Also, deficient supply of funds has hampered research into areas with limited current national prestige. Thus, models have come and gone, at times nearly lost altogether, such as the Gunn rats. The fact that they were maintained is only a miraculous accident. [The Gunn rat is a mutation of Wistar rat discovered in Toronto in 1938 and has a characteristic autosomally recessive inherited acholuric jaundice. It would have been lost were it not for the late Professor W. E. Castle, who kept a colony for 20 years, awaiting modern enzymologic knowledge. It is now known that the defect lies in the conjugation of bilirubin with glucuronic acid, and the model has been used extensively, importantly in studies of kernicterus and hereditary hyperbilirubinemia syndromes of man (9).] It is hoped that the systematic liquid nitrogen preservation of such valuable stock as embryos, feasible in mice and cattle and presumably other species, will prevent such wastage in the future.

I believe we are doing well at present in disseminating knowledge of models and in encouraging their use. More models can surely be found in exotic species—witness the cytogenetic model of the Indian muntjac (31)— but research in zoos and its support is only in embryonic stages (6). A great opportunity is not currently exploited. It requires observations first, then breeding, and finally experimentation. I believe that we are at the threshold of many new insights, paradoxically stimulated by the recognition of a vanishing wildlife.

Does the lack of standardization of possible variables, such as nutritional or environmental status, affect the reliability of intact animal experimentation?

The purist, working perhaps with clones of isolated organisms, cells, or submicroscopic particles may look askance at the possible interferences that these variables mentioned pose when working with intact animals. And, to be sure, such variables may seriously affect results. All kinds of viral and bacterial agents can infect laboratory rodents, reptiles, amphibia, and larger animals. These have been largely recognized, and the Institute for Laboratory Animals has taken great pains in disseminating knowledge about these diseases and how to prevent or treat them adequately. At least for the common laboratory animals, standards of care and housing, management, and diet have been set to minimize these variables.

This is more problematic with freshly imported animals, such as monkeys. There exists a great deal of variability in their quality and health. Almost all South American primates suffer from parasitic infections, and virus infection or the susceptibility to such infection handicaps the usage of rhesus monkeys to an extent. Breeding efficiency varies a great deal in consequence, and all would agree that laboratory-bred monkeys provide infinitely better experimental subjects than freshly imported stock (19). The trend now observed towards the establishment of primate breeding facilities will obviate these difficulties in the future. For rodents, these handicaps have largely been overcome, as quality stock, often genetically defined, is available from many suppliers. Moreover, the accreditation of laboratory animal facilities and their supervision by veterinarians in most larger research centers assure their proper management.

To be sure, there is always room for improvement. I can think of the desire to obtain dated twin pregnancies in sheep for certain work, but no pure twinning stock is yet available in this species. For some more exotic species, particularly primates, all of the nutritional and environmental standards have not yet been defined, and, as mentioned earlier, some species, such as the armadillo, cannot be bred regularly in captivity. Thus, some limitations exist in the employ of intact species, but they are becoming less problematic and do not forbid their usage.

Does the substitution of isolated organisms or tissue culture obviate or exaggerate the difficulties of obtaining dependable data that can be extrapolated to intact animals?

In my opinion, these two systems are most often complementary rather than substitutes for each other. In the examples mentioned, for instance, both procedures need to be employed side by side for the ultimate resolution of the problem. This can be illustrated by perhaps two additional examples from my own experience.

Chromosomal rearrangements are the occasional cause of sterility—at least they correlate with the infertile state (5). The rearrangement can be defined accurately in cell culture systems, but this allows no prediction of events at meiosis, which must be sampled in the adult live animal. For instance, in spider monkeys at least three different chromosomal types exist that differ by inversions and translocations (7). Only future investigations will verify or deny whether crossbreeding leads to sterility, and, it might be added, the knowledge obtained may well affect significantly the successes of breeding colonies and creation of a well-defined stock.

Thalidomide is a fairly insoluble substance, which, applied only once at the proper time, may be the cause of specific congenital anomalies. Even now, after this knowledge is at hand, it is impossible to predict at what stage in the cell cycle it has its effect. It is unknown whether the teratogenic action is perhaps due to its metabolic products. In most rodent species studied with this agent, pregnancy is not adversely affected. On the other hand, similar anomalies, as in infants, are produced when thalidomide is applied at very specific stages of rhesus monkey pregnancy (30). Testing for teratogenicity in isolated cell systems it would appear is a long way off and cannot currently substitute for animal experimentation. I am certain that other chapters in the volume will further address the possibility that *in vitro* test systems may substitute for research in intact animals.

In conclusion, this brief review indicates that *in vitro* techniques cannot be substituted for experimentation with intact animals. Enormous insights gained from such investigations have vastly improved our knowledge of human disease and improved human health and welfare. It can be anticipated from the growth of this discipline, Laboratory Animal Care, that they will be of greater variety. More genetically defined species will be needed and more dated pregnancies. Animals will have to be bred and housed to be disease free, and their manipulation will be undertaken with greater skill and concern for humanitarian principles.

Since exotic species such as primates and armadillos are now valid experimental subjects, pressure upon their wild population has mounted. Assurances must be given to create sufficient in-house capabilities of all types for their successful reproduction to meet the demand. Wider attention to other exotic species is needed. Since cell lines depend upon live animals to begin with, adequate facilities for their collection and storage must be developed lest specimens are sacrificed purely for the creation of single cell lines.

All experimental work commences with the observation, the recognition of a phenomenon. To have it become useful for successful animal manipulation, and subsequent *in vitro* delineation, the disciplines need greater communication to enhance the development of as yet unknown tools with which to eliminate disease.

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DISCUSSION

STONE: I would like to comment on questions that were asked of the last speakers. More often than not, one doesn't know whether an experiment was worth doing until after it is done; that is the nature of research.

I hope that somebody will address himself or herself to the need of animals for host-mediated assays; this is an area that is becoming increasingly important. For example, in measuring the mutagenic activity of drugs or other agents in our environment, you can do all of the *in vitro* testing that you want, but ultimately you need a host-mediated system to really evaluate the agent. This is becoming an entirely new use of animals, one that has to be studied very carefully.

STEVENSON: I apologize for being slightly off the subject, but it is a question of concern. In regard to the studies of this high-molecular testicular agent, capable of suppressing uterus formation and so forth, that is mentioned in your paper, Dr. Benirschke, would this have any relationship to the work being done by Dr. Lloyd Faulkner? And if not, would this particular method or perhaps an extract be useful for population control of animals, which is of grave concern to many of us today?

BENIRSCHKE: I don't think that the answer to this can be given at this time, until that substance, which is estimated to have a molecular weight of more than 15,000, has been identified. It is not known what the substance is, and I think it is premature to speculate about this.

Also, it would appear that this substance has different effects in cattle, where it appears to traverse through the circulation, than in primates, where it would not appear to traverse the placental circulation or is not effective in the embryo. Too little is known about this at this time. It is a very recent work.

SCHNEIDER: Mrs. Stevens, the amenities demand that I remind you that we are here by invitation of the Institute of Laboratory Animal Resources. ILAR was not born yesterday, but 22 years ago. It has been in existence all that time.

During that time, literally hundreds of biomedical scientists serving ILAR created the specialty of animal laboratory medicine, held meetings, and drafted standards for their fellow investigators. They cajoled the administrators to codify the best that they knew to improve the quality, the health, and the well-being of laboratory animals, all for the purpose of improving a study of life and disease.

Now, Mrs. Stevens, I have one question. Has ILAR helped or hindered the humane care of laboratory animals?

STEVENS: Well, thank you very much. I would say that they have done both. There is no way to give a yes or no to that one.

SCHNEIDER: We need an answer to that question. Now, either it has helped or it has hindered; on balance, if you wish, has it helped or has it hindered?

STEVENS: I would have to make a study of that, because they have done some very bad things.

SCHNEIDER: If you want to study it, please do, if you feel you cannot now extemporaneously answer this question.

STEVENS: Excuse me, Dr. Schneider, I can extemporaneously answer, but I cannot answer by yes or no. That's all.

SCHNEIDER: Well, on balance, then.

HARRELL: Perhaps she can give an illustration of each side of the point she is making.

SCHNEIDER: Yes. Would you do that?

STEVENS: Yes.

SCHNEIDER: Thank you.

STEVENS: Since I was speaking this morning about the dogs in the cages, they could have helped, and should have; on the contrary, they have actually hindered in this case.

SCHNEIDER: Who hindered?

STEVENS: ILAR.

SCHNEIDER: The record doesn't show that ILAR has any such function.

STEVENS: Well, there is a committee of ILAR that looks into such things, and they have not—

SCHNEIDER: Yes, and they drafted the standards and guidelines, and worked with the administrators trying to extricate more money from them to do that. Did you help?

STEVENS: To extricate more money? Well, I—

SCHNEIDER: Right. Did you offer to help?

HARRELL: I think I have to come to Mrs. Stevens' defense here. She has been interested in standards for cages and other aspects of animal care. She may not have worked directly with ILAR. I can't answer that question. She would have to answer that.

STEVENS: Well, I have worked with individuals who are in ILAR very often, but I have never been appointed to a committee. Now I do have the publications of the Animal Welfare Institute, *Basic Care of Experimental Animals* and *Comfortable Quarters for Laboratory Animals*, which we have given free of charge for many years to laboratories who requested them. We have been very active in that field.

SCHNEIDER: Mrs. Stevens, I am not asking you about public relations efforts. Has ILAR helped or hindered the humane care of laboratory animals?

STEVENS: And I did say that it hindered in that respect. However, I wanted to add the way in which I think it has helped. I think it has focused attention on the fact that laboratory animals need to be considered individually, by species, and that it is a serious matter that requires the National Academy of Sciences to think about it. ILAR has created the committees that I spoke of, and has had a publication that has often contained extremely valuable information. In fact, I think not too long ago, it published an article in which a decision was made that no animal that had undergone one painful surgical experiment should be used for another, unless the second one was carrying out the completion of the experiment.

I think that, for example, is a very significant thing that ILAR did. That is why I would hesitate to condemn ILAR for its faults.

SCHNEIDER: Would you praise it?

STEVENS: Certainly I would praise it for what I have just mentioned.

SCHNEIDER: Thank you.

HUMMER: I find no difficulty in responding to your question, Dr. Schneider, whether the Institute of Laboratory Animal Resources has helped or hindered the humane care of laboratory animals. My response is unqualified. ILAR has helped.

THOMSEN: Aside from the antivivisection people, nobody in the humane movement questions the need for the use of intact animals. Merely to raise the question and then take a lot of words to knock it down does not get at the problem at all. The problem is, to what extent can we substitute other models for intact animals? That is one of the questions that is before this conference. I hope that the succeeding speakers do not take our time up entirely by setting up straw men and knocking them down, but will address themselves to the basic issue here, which is to what extent can we go forward in reducing the use of animals and make the use of animals more effective and efficient.

HANLEY: In our San Francisco zoo, we have an informal ruling that our surplus zoo animals are not to be sold for research. I know that there are many other zoos that do not have that ruling. Dr. Benirschke, I wanted to know if there seems to be, in the case of animals you mentioned, such as armadillos and primates, a movement to get animals out of the zoos? Are surplus animals on a list that is circulated around to many other zoos and institutions? Is there a movement to obtain more animals from the zoos, either to do research within the zoos, such as on animal behavior, or for the betterment of zoo animals, such as what sort of living conditions should they have? Is there also a movement to use zoo animals for human research? Would that research go on within the zoos, or would those animals come from the surplus list?

BENIRSCHKE: I can answer this reasonably briefly. I think most zoos are very much conservation-oriented currently.* Most research going on in zoos is toward the better understanding and amelioration of their behavior, toward better reproduction and housing. I am aware of the surplus list. The surplus list, by and large, is one advising other zoos that "I have this animal, and if you have the female counterpart, we will be happy to have it sent to Oklahoma or wherever." So, it is an exchange program really and is not intended for the sale of giraffes or baboons, what have you, from zoos to research institutions.

I think very little, if any, of that goes on, because of the precious short supply. Most research in zoos is conducted toward the improvement of the animal health and comes, in large measure, after the death of the animals. It gives valuable insights into aging parameters, parasitic diseases, and other things.

HARRELL: I think that as Dr. Benirschke has pointed out, the number of animals available from zoo sources would be very small compared to the total number of animals used in research.

On the other hand, specific instances can be cited, such as the study of carotid blood pressures and blood flow in the brain of giraffes, where the studies have had to be done in animals of this type. Studies of that sort can be done without sacrifice of the animal.

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* For further information the reader is directed to the publication *Research in Zoos and Aquariums*. Proceedings of a symposium held at the Forty-ninth Conference of the American Association of Zoological Parks and Aquariums, Houston, Texas, October 6–11, 1973. National Academy of Sciences, 1975.

LORD: I would like to make a comment in response to a statement that was made earlier. The statement, I believe, implied that there are only 30 percent of the animal experiments that are done with medical impact, and that perhaps the remaining 70 percent are done to protect the industry. I think the speaker implied the pharmaceutical industry.

I would like the audience to recognize that there is a statute in the form of the Food, Drug and Cosmetics Act, to which the industry must comply. Part of that law deals with the establishment of the safety of drugs prior to use in human beings.

We will also have, perhaps not this year but certainly next year, another piece of legislation concerning medical devices, and again we will have to comply. We do this research for some degree of protection, but basically it is done to fulfill requirements as set forth in the law. I think this is a very important fact. We are required to do more and more safety studies to satisfy the safety of products, not only with regard to the intended conditions of use, but also under conditions of abuse. I think that the demands from the regulatory standpoint will always have to be met.

STATEMENT BY CHAIRMAN

HARRELL: As Chairman of the Organizing Committee, I would like you to know that the committee had absolutely no instruction from ILAR as to what the content of this symposium should be, nor the tone. The committee takes full responsibility.

There is no official position of ILAR on any of the subject matter that is being presented, and I believe even at the end of the symposium there will still not be an official position on any of the points discussed. All of the papers and the discussion from the floor are given as individuals and not as representatives of ILAR. To reinforce this point, I would like to introduce to you Dr. Ed Melby, who is Chairman of ILAR.

MELBY: We are delighted, of course, to have all of you here participating in this important symposium. I would point out or reiterate, as Dr. Harrell has just explained, that the program has not been orchestrated per se by an ILAR mandate. There is an Organizing Committee that has put this thing together. We thought it was a timely topic, as most of our attempts have been over the last 20 some years, to bring together groups of knowledgeable people to discuss issues, to try to identify the best thinking of the population at large here in this country, and to make this available to our colleagues in the scientific and other communities.

As such, I think the question of merit, whether or not one group has been successful versus another group is not the case in point. History always has to determine whether or not one's effort or group effort has been successful.

In the 20 some years of ILAR's activities, I think its contributions will have to be judged in historical light. Other groups, activities, and efforts, although they at some times seemingly may be divergent, are viewed in a historical light. I think the importance here is for us to be joining together, looking at mutual areas, identifying areas that need further clarification, answering questions and innuendoes, and getting on with the job of providing the very best possible care, humane care, to research animals, making the very best use of these facilities and resources for the betterment of the welfare of man.

SOL KRAMER

Ethological Contributions to the Medical and Behavioral Sciences

Animal research has become such a significant part of man's knowledge and welfare and has grown to such huge dimensions that it is now one of the characteristics of advanced countries in the twentieth century. This development of animal research in the United States, and other nations, may make regulation of the use of animals in investigations necessary. The serious concern of all of us should be that such regulation in no way impairs the development of a new, and basic, behavioral science discipline that will be of increasing importance to human health, welfare, and the stability of social systems, nations, and civilization in the coming years. I refer to ethology—a biological and evolutionary approach to the study of animal and human behavior and the considerable influence its research is having on the behavioral, social, and medical sciences.

Beforehand, however, I must indicate that when I accepted the invitation to this symposium, I had little idea in what I would be involved. As I improved my own education of this subject, I became aware of the complex and numerous problems with which we must deal. Fortunately, before coming here, I attended the Fourteenth International Ethological Conference, held in Italy, and I was able to speak with some of the ethologists from different countries about the regulations that governed their investigations. I learned about the Cruelty to Animals Act of 1876 in Great Britain and some of its benefits and difficulties. For the most part investigators are happy to have these regulations, although in some instances they either no longer appear applicable to ethological and other behavioral investigations, or the home office inspectors are not sufficiently aware of the significant research developments in the various fields, so that they may assist rather than obstruct investigations. This paper is designed to review the research methods, concepts, and contributions of ethology and to add yet another, but necessary, consideration to the problem before us.

Many of you are familiar with ethological investigations because of the remarkable job that television had done in bringing serious animal behavior studies before the general public. This would not have occurred were it not for the deep interest that so many people have, regardless of their occupation, in the basic facts and principles of animal behavior. In contrast to traditional psychological or sociological investigations, ethology represents a *biological approach* (15) to the investigation of animal behavior. The many *field investigations* viewed on television screens are an important aspect of these studies.

Ethology was initiated with Charles Darwin's chapter on instinct in *The Origin of Species* (10), in which he successfully applied the comparative methodology of anatomy to an understanding of the evolutionary origins of animal behavior. He followed this with equally illuminating studies on the behavioral selections that led to the domestication of animals (13), on our understanding of human evolution (11), and the origin of the emotional expressions, which form such a vital part of family and social behavior (12). This evolutionary approach, which revolutionized the study of biology and spearheaded many of its accomplishments in the present century, characterizes ethological research.

Although biologists were quick in their almost total acceptance of evolutionary theory as propounded by both Darwin and Alfred Russel Wallace, zoologists were uniformly slow in applying this same comparative approach to the study and understanding of animal and human behavior. They failed to realize that behavioral adaptations are similarly structured in the central nervous and neuromuscular systems and are inherited (32, 64). Consequently, for almost a century following Darwin's studies, only an occasional zoologist undertook comparative studies of animal behavior. While we cannot applaud the lay public and legislators in several of our states who, for over a century, outlawed the teaching of evolutionary theory in our public schools, we cannot rebuke them either. Zoologists who embraced evolutionary theory were equally slow in adapting evolutionary thought and concepts to all manner of behavioral and human problems. It takes not only decades, but centuries, even millennia, for a new mode of thought that can be of inestimable value and benefit to man to become part and parcel of man's activities and human institutions. We must therefore keep our officials and legislators sufficiently abreast of developments in the various fields of knowledge to prevent such legislative errors and handicaps to the advancement of knowledge from occurring again.

The following brief discussion is intended to provide the necessary prelude to a consideration of (1) how this area of investigation relates to the behavioral, medical, and veterinary sciences; (2) the implications of these findings for some of the future social problems of mankind—problems that we are only slowly beginning to recognize and to grapple with; and (3) some aspects of animal behavior study that regulations will seriously have to deal with.

THE METHODS OF ETHOLOGY

For the past three decades investigators have begun to study animal and human behavior, not from psychological, sociological, and anthropological viewpoints, much as these disciplines have and will continue to contribute to our knowledge, but from a biological orientation. Simply stated, this affirms that all animal behavior, including human behavior, has both an evolutionary, ancestral basis and an individual developmental history. Although ethologists employ biochemical, neurophysiological, and all manner of sophisticated technological devices in their analyses, the evolutionary origin of behavior remains a prime consideration.

The core of the biological approach to the study of animal behavior resides in the fact that, just as the life functions of higher plants and animals are based on the structure and function of cells, so the behavior of all animals with a nervous system is based on genetically determined neuromuscular coordinations, or movement patterns. The inability of neurophysiology to provide a biological foundation for psychiatry, for example, rests specifically on the failure of this discipline to recognize the biological fact that animals are unable to move individual muscles, let alone a motor unit (a single nerve fiber and the muscle fibers it serves). Nervous systems only can initiate patterns of movement. Ethologists have termed these units of behavior *fixed motor patterns* in recognition that they represent inherited, not learned, units of behavior, which are structured in the neuromuscular system of animals and are subject to evolution.

The observations and experiments of ethologists are often derived from the study of a species, or related species, in nature. Such comparative studies, particularly when the species originate in widely separated geographical areas, strongly emphasizes the genetic aspect of the behavior patterns observed. Captured species used for study are kept under seminatural modes of confinement designed to encourage normal aspects of the behavior repertoire. As much as possible, the investigator endeavors to accumulate a description of all the motor patterns in the species' repertoire—the *ethogram*.

Very often investigators start developing an ethogram from animals kept in confinement or in zoos and then utilize such partial ethograms as a basis for additional studies in the field. Where aspects of social behavior are under scrutiny, as with primates, these may be confined in large field enclosures, as occurs in some of our regional primate centers. Or zoo observations may provide the basis for field experiments to confirm a suspected inherited tendency of chimpanzees to utilize clubs or rocks in defense against predators. Experiments in such "field laboratories" will increasingly take place alongside experiments of caged animals.

One of the questions that confront ethologists deals specifically with the "innateness" or inherited aspect of behavior. Sauer (61), who isolated and raised whitethroats (*Sylvia communis*) in soundproof chambers, found that they had developed and sang all 25 species-specific songs. Konishi (36, 37) endeavored another approach to this question. By means of surgical procedures, he deafened chickens at a very early age and found that they still were capable of making their species calls. Similar surgical procedures were utilized to deafen juncos, grosbeaks, and robins, with the same result—they all sang their species-specific songs. Nicolai (53), on the other hand, demonstrated that young bullfinches simply learn the songs of their parents. A young bullfinch raised by canaries sang like a canary.

These field investigations, utilization of animals in seminatural environments and in zoos, as well as isolation experiments, surgical procedures, and the use of foster parents, are meant to suggest the range of experiments that ethologists engage in when they investigate animal behavior. The existence of inherited patterns of behavior in the nervous system of man and animals is of considerable theoretical and practical importance. It should be clear that ethologists and other investigators have utilized a variety of methods of housing and studying animal behavior, as well as a diversity of techniques in their research. All these methods will have to be considered when regulations are contemplated.

INNATE AND LEARNED BEHAVIOR

Animals without a nervous system, such as the amoeba and paramecium, are capable of adaptive behavior patterns, as Jennings (34) so ably demonstrated. The paramecium can swim in a near straight line toward favorable stimuli; it can direct streams of water containing food into its gullet from a distance. It can thus "sample" a distant environment and respond before it enters that environment. After avoiding an unsuitable environment, it can test alternate paths of movement and choose the most favorable new direction. The amoeba can similarly react adaptively to chemicals, light, heat, electricity, and other factors in its environment. After several forays into a bright-light environment, it learns to reverse its direction as soon as the tip of one of its pseudopods enters strong light (50).

Thus, even without a nervous system, these and other elemental animals possess both innate, adaptive patterns of movement and response and the capacity for learning. As more complex animals possessing specialized sensory mechanisms evolved, a muscular system developed for the facilitation of movement and other behavior, integrated by an increasingly complex nervous system. Subsequently, within the nervous systems of all animals there emerged, through maturational processes alone, complex and completely integrated patterns of movement and behavior that adapted them to their environments. These behaviors are integrations of the neuromuscular system, and are, in fact, structural adaptations. No one will take issue with the fact that behavior is structurally based in the nervous system, but this statement has a very general connotation in contrast to the recent ethological concepts that such behavior is very *specifically structured* in the nervous system.

The larval tiger salamander is fully capable of swimming, as soon as it emerges from 48 hours of development and maturation in the egg. The frog can catch flying insects on land after it metamorphoses from a tadpole. The chick can peck at and swallow grains of food a few hours after emerging from its eggshell. All these animals have the neuromuscular structure necessary to support such behavior.

We were so impressed with these instinctive or inherited patterns of behavior in animals, that we tended to overlook the fact that all animals since the amoeba have retained and developed, in varying degrees of complexity, through their nervous systems, the capacity for learning. On the other hand, in the animal man, we were so impressed with his remarkable capacities for learning that we tended to depreciate the fact that man's nervous system also contains all the motor patterns of his primate, mammalian, and vertebrate ancestry. Academic psychology, neglecting evolutionary theory, endeavored, with varying degrees of success, to convince us that Darwin's instincts were a figment of his and our imaginations, and that man, and other animals, behaved as they do as a result of learning, conditioning, or reinforcement. The significance of the comparative, evolutionary approach to the study of behavior was recognized at the start of this century by such men as C. O. Whitman (73, 74), W. M. Wheeler (71, 72), and O. Heinroth (30), but zoologists neglected their pioneer paths for over half a century.

Largely through the work of Karl von Frisch (25), Konrad Lorenz (45–47), Niko Tinbergen (68), G. P. Baerends and J. M. Baerends-van Roon (4), Otto Koehler (35), William Thorpe (67), and their students, this one-sided view of animal behavior was challenged and shown to be both limited and erroneous. The discipline of ethology has, during the past several decades, been established as an important area of biological, behavioral research. Numerous laboratories, institutes, and departments of ethology and animal behavior have been established in England, France, Germany, the Netherlands, Italy, Australia, and other countries that support biological behavioral research with animals. The development and recognition of ethology has been slower in the United States, but it has, nevertheless, had a tremendous influence and stimulation of animal behavior research in this country among anthropologists, psychologists, sociologists, and zoologists.

The European Association for the Study of Animal Behavior has a membership of over 600, and the affiliated Animal Behavior Society of North America has 1,700 members. The Fourteenth International Ethology Conference, held in Italy and from which I recently returned, was attended by some 400 participants, almost half of whom can be classified as recent investigators into the ethological field.

FIXED MOTOR PATTERNS

Perhaps the best introduction to the concept of fixed motor patterns would be to take you back with me to the Max Planck Institute for Behavioral Physiology in Germany, where the field laboratory is a small lake outside the buildings (Figure 1A). Some 15–18 or more species of ducks and geese (Anatidae) are "kept" and studied. The ducks and geese are free to behave as they would in nature, even to fly off. They remain and make this their home, however, because they are well fed daily. The food supply keeps them "at home." The birds are marked and named, and their individual behaviors and life histories are recorded.

They are more or less habituated to people, and, although they still keep their distance, it is short enough so that they may easily be observed. One aspect of their behavior frequently seen is the wing–leg stretch, which consists of stretching one leg and one wing at a time on the right or left side of the body. Half the tail feathers on this active

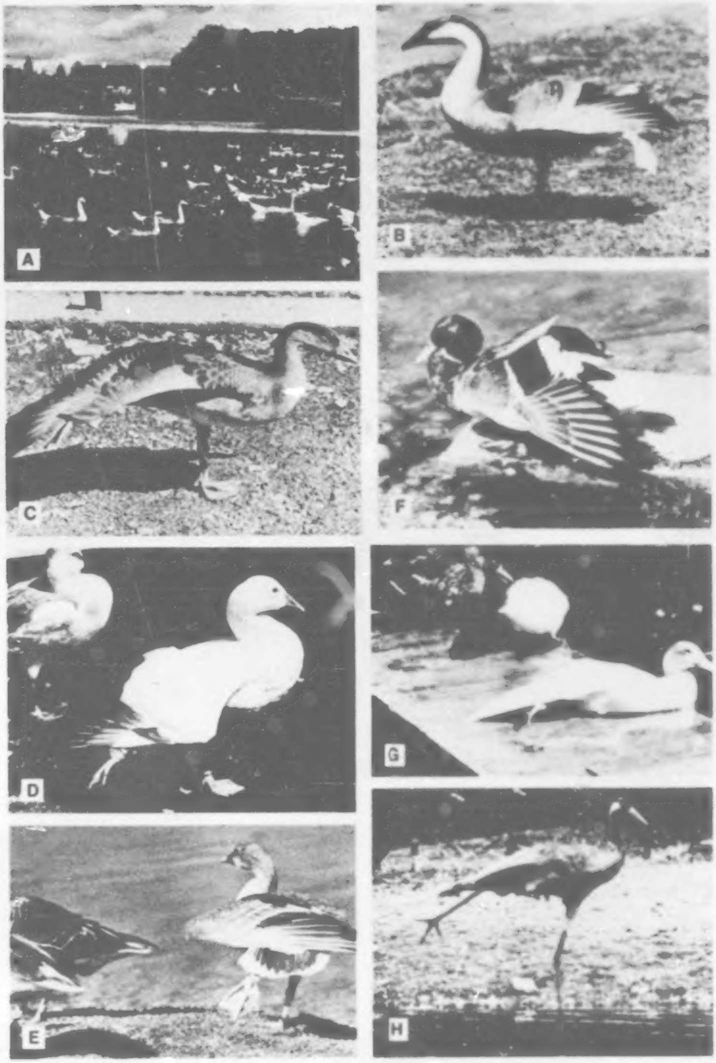

FIGURE 1 (A) A "field laboratory," Lake Seewiesen, Max Planck Institute for Behavioral Physiology, Starnberg, Germany. Wing-leg stretches in (B) Chinese swan goose (*Anser cygnoides*), (C) greylag goose (*Anser anser*), (D) snow goose (*Chen caerulescens*), (E) pink-footed goose (*Anser brachyrhynchus*), (F) mallard duck (*Anas platyrhynchos*), (G) seated, albino mallard duck, (H) crane (*Grus grus*). Author's photographs.

body side are also stretched. Figures 1B–1H show this stretch movement in a Chinese swan goose, a greylag goose, a Canadian snow goose, a pink-footed goose, two mallard ducks, and a crane. These birds are normally found in different parts of the globe, yet they all have the same neuromuscular coordination in their nervous systems. Further evidence that it is an unlearned bit of behavior is the fact that newly hatched goslings and ducklings perform these stretching movements within a few hours after emerging from their eggs.

All birds have these stretch movements in their nervous systems, and it is particularly amusing to watch the red-crowned crane attempt it. Because of its long, stiltlike legs, this stretch movement is quite a balancing feat. I have watched it make three and four unsuccessful tries before completing the movement, while standing on one leg. If any learning was involved in this stretch movement, one would think that the crane would learn to stretch first the wing, then the leg, instead of always endeavoring to stretch them together in spite of repeated failures. Simple as these stretch reflexes are, they are also genetically determined neuromuscular coordinations—the result of maturation, not learning. All fixed motor patterns are similarly patterns of specific movement residing in the nervous systems of animals.

THE LAWFULNESS OF BEHAVIOR

Numerous investigations and experiments have demonstrated that the fixed motor patterns (FMP's) of animals are released by certain specific sign stimuli, or releasers (R) in the animals of their species or in the environment. The reproductive behavior of the stickleback, which occurs in the spring of the year, consists of a hierarchical system of fixed motor patterns (68) that have never been performed before. It is comprised of (1) migration from brackish to fresh water; (2) establishment and defense of a territory by the male; (3) building and repairing a nest, courting and spawning with several females; and (4) defending the school of stickleback fry when they start swimming 8–9 days later. All these unlearned behaviors are based on a series of fixed motor patterns and releasers.

For example, courting and spawning with a female stickleback that enters the male's territory is characterized by: the absence of a red belly in the female (R); inhibited attacks on the female, which becomes the zigzag courtship dance (FMP); the male leading the female to the nest (FMP and R); the female following the male to the nest (FMP and R); the male pointing at the nest (FMP and R); the female entering the nest (FMP and R); the male nudging the caudal fin of the female (FMP and R);

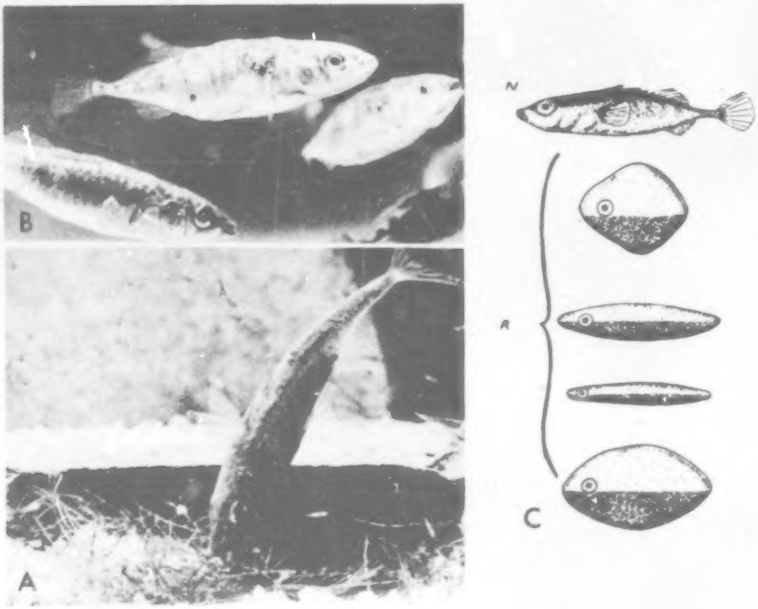

FIGURE 2 (A) Male, three-spined stickleback (*Gasterosteus aculeatus*) tending his nest of just-hatched fry. His darkened belly is red. (B) Females with egg-enlarged, white bellies. (C) Cardboard dummies used to test aggressive responses of male. Printed with permission from the Clarendon Press, Walton Street, Oxford, from *The Study of Instinct* by N. Tinbergen, 1951.

the female spawning, then leaving the nest and territory (R); and the male entering the nest, depositing his sperm, and also leaving the nest. He may later repeat this behavior with one or two additional females that enter his territory.

All red-bellied males (Figure 2A) that enter the territory are attacked and driven off, while white-bellied females (Figure 2B) are courted. This has been determined by experiments with cardboard dummies, which are placed in the male's territory, and noting his responses (Figure 2C). The dummies that look exactly like a stickleback but have a white belly are rarely, if ever, attacked; but those that have round, oval, and other shapes and look nothing like sticklebacks, but have red or purplish-red bellies (R), are regularly attacked. In the courtship and spawning series mentioned, both color signals and the motor patterns of the partner act as releasers. This behavior between male and female, based on motor patterns and releasers, is both "reproductive behavior" and "social behavior." The social behavior of all animals is governed by such motor patterns and releasers.

Another experiment to demonstrate releasers has been done with the oystercatcher (Figure 3). If an oystercatcher that is brooding its own small egg is presented with a giant dummy egg that has the exaggerated markings of its own egg, it will leave its own egg and brood a larger herring gull egg or an even larger dummy egg made of wood. Likewise, a mounted, immature robin with brown breast feathers will rarely be attacked in a male robin's territory, but a mere tuft of red feathers, characteristic of adult males, will be threatened immediately. Releasers have been demonstrated for a variety of animals and their mutitudinous individual and social behaviors.

This quick sketch of fixed motor patterns and releasers has one aim: To impress the fact, and this is one of the salient contributions of ethology, that all behavior in the animal world is not learned. Within the central nervous system there must therefore develop, as part of the normal processes of maturation, mechanisms that inhibit certain patterns of behavior from taking place and subsequently excite them when the appropriate sign stimulus or stimuli appear. Ethologists have termed this hypothetical, and still to be discovered, mechanism the *innate releasing mechanism*.

Another concept emerging from ethological investigations is that behavior can no longer be strickly viewed as either being "random" and amenable to all and sundry modifications that we wittingly or unwit-

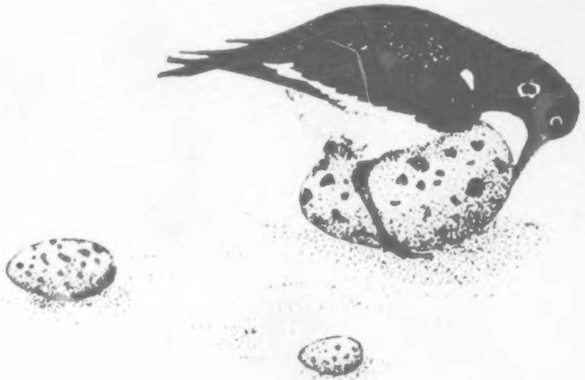

FIGURE 3 Oystercatcher attempting to brood a giant egg in preference to its normal egg (foreground) and a herring gull's egg (left). N. Tinbergen, 1951.

tingly decide to make or a completely chance occurrence in an animal. Just as there are laws governing the forces that underlie physical and chemical reactions, so have there been demonstrated laws that regulate the kinds of behavior that emerge from animals presented with a variety of stimuli in diverse environmental situations.

In the type of behavioral experiments that have normally been conducted in a laboratory setting, the experimenter has endeavored to keep all other conditions constant, while a single stimulus has been varied. In nature, and especially in social situations, animals are subject to stimuli that might elicit more than one behavioral response. The discovery of releasers had provided ethologists with unique experimental opportunities. It now becomes possible to combine two or more releasers and study the effect of this summation, to present an animal with releasers for two different motor patterns and study what happens when such an internal "conflict" is excited within the central nervous system, or to provide an animal with a single object containing releasers for two different types of behavior. For example, nesting herring gulls will brood white dummy eggs, but will carry red-colored objects (usually the insides of broken, hatched eggshells) away from their nests. If we paint a dummy wooden egg red and place it in a nest of the herring gull, it will first carry away the red dummy wooden egg (red-colored object), then sit down and brood the dummy, then carry off the red object, and so forth. In this way, it is possible to study "ambivalent" behavior, which we normally regard as being "psychological," at the perceptual-motor level. The emphasis of ethologists in laboratory or field experiments is on complex, or varied, patterns of behavior designed to simulate the natural life of the animal.

Ethologists have developed various concepts, hypotheses, and theories associated with the ways in which such motor patterns function in the central nervous system. In one kind of typical conflict situation, neither of the normally released motor patterns occurs, but a third, seemingly irrelevant, pattern appears. This is termed a *displacement activity* (3, 38, 69). Such displacement activities very often evolve into signals, or "body language," which other members of the species appear to "understand" and react to accordingly. Thus, the displacement sand-digging of the three-spined stickleback serves as a threat signal to other sticklebacks at the territorial boundary of a reproductive male, and other males appear to heed this communication by not encroaching on the territory of the male displaying it.

All neuromuscular patterns of activity are accompanied by autonomic nervous system patterns, which normally support the particular behavior an animal may be engaged in at a given moment. Morris

(51) and others have shown that in conflict situations not only do neuromuscular displacement activities occur, but also new autonomic patterns as well, which result in modified pilomotor, cardiovascular, respiratory, or other responses. If these autonomic patterns develop into signal functions, they often become exaggerated in the course of evolution or combined with structural modifications that make these responses readily visible to other animals. Therefore, not only neuromuscular patterns, but also displacement activities and displacement autonomic patterns of response, are subject to intensification or exaggeration through selection and are capable of evolving into communicative signals or releasers of social behavior in animals. The respiratory hissing of snakes, the hissing and spitting of cats, even of their blind kittens when only a few days old, the urinary territorial markings of dogs and wolves, and the pilomotor and vasomotor signals of birds and mammals all fall into this category.

There appears to be a continuous spectrum of responses in the nervous system from the simplest inherited reflexes of animals to the more complex sequences of motor patterns that comprise a functional behavioral act. Whenever the stimulus for a complete response is of low intensity or is combined with the lowered physiological state of the animal, various incomplete behavioral sequences of an action chain occur. For example, prior to initiating the normal pattern of flight, a bird will bend its legs, stretch its legs, or flutter its wings. Sometimes the bird will perform these preliminary flight movements without taking off. Such movements indicate an intent, or incomplete inpulse, to fly. These preliminary flight movements are designated as *intention movements.**

Another behavioral phenomenon observed in animals is the combination, or *superposition*, of two separate motor patterns into a new behavioral expression. For example, an aggressive goose will extend its neck, while a fearful one bends it back or retracts it. When the superposition of these two motor patterns results in an extended, partially bent neck, which frequently occurs, it indicates a mood of fearful aggression. Dogs, primates, and other animals similarly superpose the two responses of fear and aggression into several nuances that have evolved into new expressions.

Fixed motor patterns normally associated with an earlier stage of development may be reactivated, not only in other animals, but also in man. A child who has been walking bipedally for months, or even a year, sometimes reverts to crawling in the presence of an infant sibling or when his mother fusses admiringly over a neighbor's infant. In LSD psychotherapy 30-, 40-, and 50-year-old patients exhibit sucking reactions of such intensity that the therapist feels obliged to provide an object or even a hand for the patient to suck on (40, 48).

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* It should be noted that while the all-or-none law of physiology holds true for the motor unit (a nerve fiber together with the muscle fibers it supplies), it does not hold for sequences of motor patterns. In given nerve bundles, only a small number of nerve fibers may be "recruited," which then only partially activate a given sequence. This latter undoubtedly provides the basis for intention movements.

As part of the bonding ceremony of herring gulls, the female begs for food from the male with the same posture and begging movements that herring gull chicks use with their parents. The male, in fact, often responds to this behavior by regurgitating food and feeding her, much as he would respond to the releaser of a food-begging chick. Male pigeons feed their females in the preliminary courtship that precedes copulation. Adult dogs and cats resume their previously recorded puppyish or kittenish behavior after becoming neurotic. A similar return to earlier modes of behavior is part of normal, human sexual behavior.

These are all examples of *regressive behavior*, which we have heretofore so exclusively regarded in a psychological context that we have overlooked the role of the neuromuscular system in these activities. At the very least we must consider the biological fact that the neural mechanisms responsible for such behavior remain present in the central nervous system, regardless of conditioning or "socialization," and are always subject to reactivation.

We cannot think of regression as being exclusively an undesirable mode of behavior. Kortlandt (38) points out that, just before juvenile cormorants in Holland are able to migrate south in the fall, they reactivate the parental relationships from which they had previously separated themselves and return to juvenile food-begging for a day or two before flying off toward an unknown destination in Africa. When presented with an unknown or fearful object or situation, infant rhesus monkeys and children alike return to clinging to their surrogate or real mothers before exploring the new object. On the adult human level, the capacity for intimacy is correlated with the ability of sexual partners to revert temporarily to infantile modes of expression and sexuality. Fried (23) suggests that some psychotherapists are so preoccupied with strengthening the patient's ego, that they fail to see and support the regulatory function of passing phases of ego regression.

Another behavioral phenomenon that occurs with regularity in the dominance hierarchies of various groups of animals is *redirected activity*, first called to our attention in the peck order of chickens. When a higher-ranking chicken drives a lower-ranking chicken from the feeding area, it activates the latter's aggressive behavior, but past experience with the dominant animal inhibits its aggression and it attacks and pecks at a lower-ranking chicken that happens to be passing by. The nervous systems and behavior of fish, birds, mammals, and man have been shown to react in an equally lawful way under similar circumstances. In man such behavior is equivalent to the concept of "object displacement" in psychoanalysis.

All these behavioral phenomena, such as displacement activities, ambivalent responses, intention movements, superposed movement patterns, regressive behavior, and others, are the result of lawful functions within the nervous systems of animals. Together with the basic motor patterns from which they stem, they have been shown to be responsible for the many signalling or communicative systems that play such a vital role in the evolution of social behavior.

IMPRINTING

Another example of behavior dependent on inherited tendencies is *imprinting*, seen in Figure 4A, which depicts a flock of Chinese swan goslings swimming after "mother goose." Experiments have shown that many newly hatched birds, especially waterfowl, have an inherited inclination to follow moving objects, and, once they follow (normally their own parent), they learn to distinguish and follow their parent from all other members of their species.

Figure 4B depicts mallard and Muscovy ducklings imprinted on an Egyptian goose. These ducklings had an opportunity to socialize with mallard and Muscovy ducks as they matured. When the males became sexually mature, however, they went about the area apparently searching for Egyptian geese to mate with. Schutz (62, 63) has since demonstrated that there is a period of "sexual imprinting" as well as parental imprinting. Female mallards apparently recognize male mallards innately, but males will court the species with which they have been raised. If male mallards are raised only with males, they will form "homosexual pair bonds" with other males.

Figure 4C depicts a mallard duckling imprinted on a boxer bitch that had not had a litter in several years. Not only did the duckling follow the boxer, but she in turn licked and defended the duckling.

Figure 4D shows a human-imprinted greylag mounting the hand of Konrad Lorenz and attempting to mate with it, even to the extent of grasping his raised finger and plunging it underwater. During copulation the gander frequently plunges the neck and head of the goose underwater. Though some refer to imprinting as early learning, it

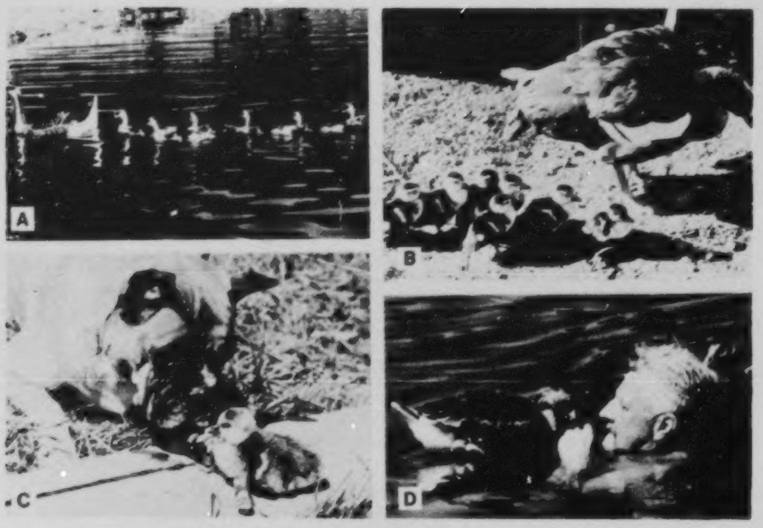

FIGURE 4 (A) Imprinted goslings following Chinese swan goose mother. (B) Mallard and Muscovy ducklings imprinted on Egyptian goose *(Alipochen aegyptiaca)*. (C) Mallard duckling imprinted on boxer bitch. (D) Greylag grasping finger of Konrad Lorenz and attempting to copulate with him. Author's photographs.

differs from ordinary learning in several respects. There is an early sensitive, or critical, period in the life of the young animal during which this tendency to learn or respond to a stimulus is very strong. Hess (31) demonstrated that in the mallard duckling this tendency to follow, present during the first 35 hours of life, is strongest from 13 to 16 hours of age, but fades very rapidly after this period (Figure 5).

The above are examples of object imprinting, but there also exist "motor-pattern imprinting," wherein chaffinches exposed to the song of their parents learn the song long before they themselves are able to sing. The general pattern of the song is innate, but Thorpe (67) has shown that the details of the song may be quickly learned, or imprinted, if the young birds once hear the chaffinch pattern, to which they selectively respond. Other forms of imprinting and early learning periods in animals are reviewed by Sluckin (65).

THE EVOLUTION OF SOCIAL SYSTEMS IN ANIMALS

Tinbergen (69a) considers the study of social behavior as the study of cooperation among individuals and defines social behavior as occurring when two or more animals of the same species interact with each other. As few as two animals may be involved, as in courtship and reproduction. Thirty or more birds may take part in the predator-mobbing response of a reproductive "club" of herring gulls, or thousands of individuals may cooperate in the flight and social roosting of a flock of starlings.

It is now apparent that fixed motor patterns and the lawful phenomena associated with them have played a central role in the evolution of whatever cooperative social interactions and social structures we find in animals. Or, put another way, all animal social systems, including those of man, are fundamentally biological in nature and dependent on an array of motor patterns structured in their nervous systems. If we hope to deal more rationally with the evolution and changes that are certain to take place in human social systems during this and future centuries, then we will have to understand the evolutionary origins and biological dynamics of the social system within which we ourselves are living.

A consideration to be emphasized is that the very cooperative nature

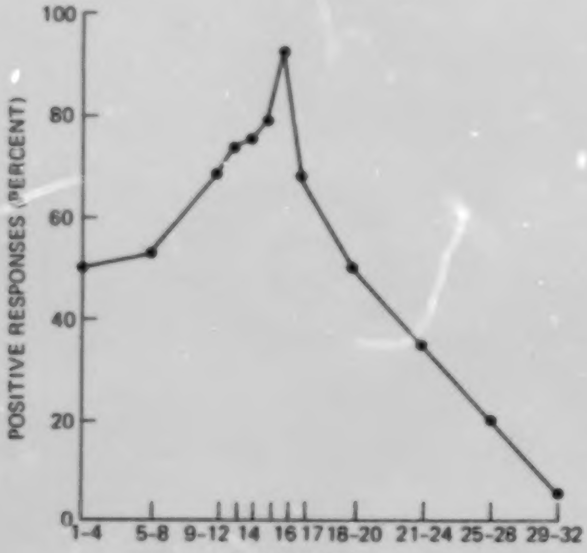

FIGURE 5 Peak, critical imprinting period for mallard ducklings tested for following responses at various ages (in hours) after hatching from the egg. After E. Hess, 1959.

of social behavior and structure is founded on conflict. The term "conflict" is used to describe several diverse types of biological responses and interactions, but they may be grouped as follows: (a) intraindividual conflict, (b) conspecific conflict, and (c) interspecific conflict. All three categories of conflict have resulted in the evolution of mechanisms that contribute to or enhance social behavior.

Intraindividual Conflict

Intraindividual conflict refers to a conflict of responses within a single animal, such as approach or avoidance in courtship rituals, flight or aggression in fighting for a territory, and so forth. This internal conflict of antagonistic motor patterns gives rise to ambivalent behavior, or to the displacement activities previously mentioned. Since first described by Kortlandt (37a), many ethologists have called attention to and described the regular and predictable occurrence of displacement activities in a variety of animals. The feather preening of birds, the scratching of mammals, and so on, are frequent displacement activities occurring in many species, which may evolve into social signals as the result of conflict situations between two individuals of the same species.

Tinbergen (69) suggested that displacement activities may provide outlets for surplus impulses that might disturb or otherwise damage the central nervous system—that is, that they have a homeostatic function. Van Iersel and Bol (33) carried out a quantitative analysis of displacement feather preening in two species of terns (*Sterna sandwichensis* and *S. hirundo*). Preening is a fixed motor pattern that normally occurs after bathing, but displacement may occur in terns after copulation, after an alarm signal, during nest-relief ceremonies, during brooding, or in connection with aggressive displays. We still know practically nothing about the underlying neurophysiological mechanisms that account for the choice of a specific motor pattern. Why, for example, does displacement preening occur in some instances and displacement head-shaking in others? Analytic and other studies in the coming years will provide more insight into the operation, predictability, and function of these neurophysiological mechanisms and their relation to the general welfare of animals and human health.

From the above studies, a conflict situation has been defined as one in which two opposite systems of overt motor activity are simultaneously activated but mutually inhibit each other. There are other types of situations, however, as in the courtship of the stickleback fish, when displacement activities occur. When the female suddenly stops following a courting male, the latter may start fanning behavior with its gill fins (displacement fanning); or, if one of two fighting domestic cocks suddenly flees or is artificially removed, the remaining one may begin displacement food-pecking. All such behavioral relationships are based on visible, gross behavior, but it is certain that both the conflict of responses and their displacement activities must involve lawful, and as yet little-understood, central nervous system mechanisms.

In the human realm, we often group this and similar types of internal conflict under the heading "emotional tension," resulting from incompatible inner needs or drives. It should be noted that muscle tension is a valid physiological phenomenon, and emotional responses always involve underlying fixed motor patterns. These emotional neuromuscular patterns are an expression of the autonomic nervous system which Gellhorn and Loofbourrow (26) have experimentally verified as lying at the heart of mental illness and psychiatric problems. A succeeding section will deal with the fixed motor pattern's role in human behavior, personality formation, and psychosomatic responses.

Conspecific Conflict

When two or more individuals of the same species fight for food, a territory, shelter, dominance, a mate, or even threaten each other, we have examples of conspecific conflict. Conspecific conflict and intraindividual conflict are often dynamically interrelated, for frequently when two individuals of the same species oppose, court, or cooperate with each other diverse internal patterns of response are activated that are in conflict within the nervous systems of the animals.

If two pairs of *Tilapia sparrmani* have territories at opposite ends of a 20-gallon aquarium, open-mouthed threats occur between opposing members of the pairs. The two fish stop for this ritual within 1 or 2 inches of each other. Not infrequently, these threats are followed by vicious attacks and fights. Male *Tilapia mossambica* in adjacent spawning territories regularly threaten each other in open-mouthed fashion at the territorial boundary, but they rarely attack each other. We have already noted how the conflict between aggression and fear within the stickleback, resulting in displacement sand-digging, has evolved into a social threat signal between male members of this species. Such ritualized patterns of behavior have evolved again and again among animals and enable them to defend or fight for territories without killing or harming each other.

Interspecific Conflict

The attentive raised head, which a goose displays at the first sign of a predator, has evolved into an alarm signal that other geese in the area immediately respond to with similar stiff-necked raised heads. In a few seconds all members of a colony are alerted and ready to fly off.

In the African Thomson's gazelle, a raised neck and head of alarm is often accompanied by a twitching of the flanks. This twitching of the flanks is normally associated with body-care movements, such as when flank-twitching follows scratching the neck with the hind legs. When a bachelor gazelle enters the territory of another male, he is confronted by the territory's owner. The bachelor usually lowers his head and turns alternately right and left, stamps the ground with all four legs, pushes his nose against his inguinal region, scratches his head or neck, and then shakes his flanks (70).

These latter are body-care displacement activities that occur during this conspecific conflict. During a predatory interspecific-conflict situation, all the initial sequence of body-care movements drop out, and displacement flank-twitching alone occurs. The American pronghorn and whitetail deer behave similarly, except that the latter's flank contains a fluff of white hairs, which emphasizes the alarm signal. It appears that aspects of intraindividual conflict, conspecific conflict, and interspecies conflict were all incorporated into the evolution of the social alarm signal of Thomson's gazelle and other deer.

Similar dynamic and lawful neuromuscular mechanisms have been shown to be involved in the social evolution of colonies of reproductive birds (gulls, terns, cormorants, pelicans, and so forth), herds of mammals (deer, sheep, goats, elephants, fur seals, and others), and packs of hunting mammals (such as wolves and hunting dogs), as well as primates. These, and the concepts that follow, are summarized in Figure 6.

SOCIAL SYSTEMS IN PRIMATES AND MAN

We now have abundant evidence from diverse disciplines that the mother–infant relationship, together with the extended periods of infancy, juvenile development, and adolescence, represents a crucial developmental phase for the foundation of health and social structure in all primates, including man.

The development of psychoanalysis was based on phylogenetic hypotheses, as well as on psychogenetic and ontogenetic considerations of numerous case histories of so-called mental disturbances

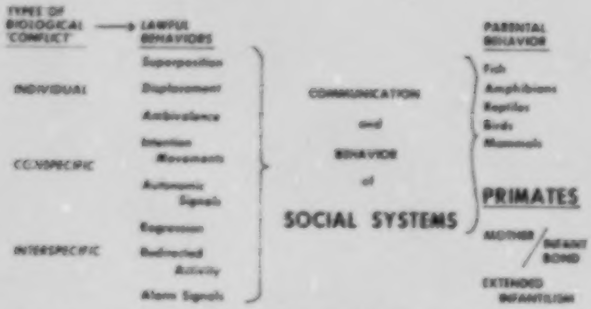
TYPES OF BIOLOGICAL CONFLICT
LAWFUL BEHAVIORS
INDIVIDUAL
CONSPECIFIC
INTERSPECIFIC
Superposition
Displacement
Ambivalence
Intention Movements
Autonomic Signals
Regression
Redirected Activity
Alarm Signals
COMMUNICATION
and
BEHAVIOR
of
SOCIAL SYSTEMS
PARENTAL BEHAVIOR
Fish
Amphibians
Reptiles
Birds
Mammals
PRIMATES
MOTHER / INFANT BOND
EXTENDED INFANTILISM

FIGURE 6 The lawful behaviors, which arise out of biological conflict situations and mediate social systems throughout the animal kingdom.

that medicine was previously unable to understand and adequately treat or alleviate. Through the decades of this century, we have slowly returned to looking at man's behavior and health from an evolutionary perspective. In the psychiatry departments of our medical schools, we are beginning to approach mental and behavioral disturbances with psychoanalytic, psychodynamic, ontogenetic, and primatalogical orientations, as well as with consideration of psychobiological factors and sociocultural issues in treatment procedures. Notwithstanding these developments, and the development of so many phases of psychosomatic medicine (which experimentally demonstrated the intimate relationship between psychic, physiological, and biochemical mechanisms), our medical schools still remain divided into "hard-core" physiological, biochemical, internal, orthopedic, and surgical medicine and "soft-core" psychiatric or psychodynamic medicine, even though considerable input is made by behavioral science departments in some medical schools.

This situation persists largely because of (1) a neglect of the evolutionary aspects of the biological basis of medical practice and overemphasis on purely functional biology in the medical curriculum, (2) a neglect of the functioning and physiology of the neuromuscular system in human development, and (3) the failure to appreciate that *socialization and personality development represent a biological modification of man.*

The fixed motor patterns of ethology supply the objective tool we need to return us to an evolutionary and more accurate consideration of the process of socialization, which underlies human development and provides a biological foundation for dealing with human personality as it applies to health and illness. It is to this area that I now turn.

MENTAL ILLNESS AND DEGENERATIVE DISEASES IN MEDICINE

The concept of human health, disease, and illness has undergone many changes through the centuries. Although empirical medicine began much earlier, great progress was made when it accepted the demonstration by a French chemist (Louis Pasteur) that microorganisms could be responsible for the ropy taste of wine, the souring of milk, and rabies. Pasteur's work (14) initiated bacteriology and aseptic surgery. Yet these very breakthroughs, and the thinking they initiated, beneficial though it proved to be, interfered with the discovery and demonstration that a mosquito vector could carry the organisms responsible for malaria. Such thinking also hampered the discovery and acceptance that the lack of chemical substances—vitamins—could be responsible for scurvy, beriberi, and other diseases. We are slowly beginning to accept the fact the disturbances of function, such as those responsible for the degenerative diseases or illnesses—heart attacks, stroke and other cardiovascular breakdown, kidney and liver dysfunctions, intestinal tract dysfunctions, and other disorders—may be due to causes other than pathological microorganisms. We have all but forgotten that disease refers to any impairment of the normal state of the living animal that affects performance of vital functions, whether the etiological factor is infectious, metabolic, toxic, genetic, psychosomatic, degenerative, traumatic, or a disturbed personality.

If personality disturbances prevent the performance of adequate parental behavior, now recognized as a vital aspect of child development, then shouldn't we speak of mental diseases? We prefer, however, because of previous connotations of insanity and asylums (forgetting that this once denoted a place of retreat, refuge, and security), to use terms like mental disorders, mental illness, and mental health. In the present inability of medicine to find an adequate descriptive category for these disturbances of behavior, we tend to overlook the fact that the numbers of those who seek psychiatric or psychotherapeutic help, and who fill our psychiatric wards and mental institutions, are annually increasing at a rate out of proportion to the population increase. Admittedly, a lessening of resistance to seeking help may be a factor in this increase.

However we classify or explain them, the overwhelming majority of these mental disorders are personality disturbances that interfere with interpersonal, parental, family, and social behavior and with work, or developmental disturbances that make normal personality formation almost, if not totally, impossible. Medicine, and the lay public, has only gradually recognized these disturbances of personality, which we have come to understand psychoanalytically and psychologically.

I have pointed out elsewhere (40, 41, 42) that personality or character structure refers to a biological, physiological, and structural modification of man. Biologically, man may be said to be a neuromuscular phenotype. We have learned from psychoanalysis, as long known to primitive religious systems, that the fundamental animal, emotional, sexual, and aggressive drives are present in man. The core of psychoanalytic theory, as well as the psychoanalytic concept of character, centers around the suppression* of these drives, or animal instincts. If we wish to understand how this suppression is accomplished biologically, we must turn to Darwin's *The Expression of the Emotions in Man and Animals* (12).

Largely unnoticed by biologists and psychoanalysts alike, Darwin provided the neuromuscular basis for the suppression of behavior when he analyzed how the continuous impulse to cry, coupled with the desire to inhibit this expression, led to the evolution of the expression of grief. Crying involves both the impulse to cry out, which many young animals do when separated from their mother, plus the reflex closing of the eyes. This latter reflex accompanies any violent expiration, such as screaming, coughing, or sneezing.† The fixed motor pattern of crying involves a large group of muscles, which includes the zygomatic muscles, the labial levator muscles, the mouth angle depressors, the quadratus (for the scream), the orbicular muscles of the eyes, the nose pyramidal muscle, and the eyebrow corrugator muscles (for closing the eyes). To stop crying, a child attempts to keep his eyes open, but to do so he must prevent the contraction of the orbicular, pyramidal, and corrugator muscles. He accomplishes this by a contraction of the central fasciae of the frontal muscles of the forehead. In so doing he raises the eyebrows at an oblique angle, part of the characteristic expression of a grief-stricken person. Similarly, when a child tries to look at an object against the background of a sunny sky, his eyes begin to close reflexly, but, in his attempt to keep them open so as to focus on the object, he also contracts the central portion of the frontal muscles. In this case, too, an interaction of two sets of motor patterns takes place in the suppression of this behavior.

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* The term "suppression" normally is applied to a conscious, voluntary inhibition of behavior, whereas the term "repression" refers to a secondary unconscious inhibition of the thoughts and ideas associated with or giving rise to such behavior.

† The reflex closing of the eyes is apparently an adaptive mechanism that allows the ocular fluids to counteract the increased blood pressure in the retinal capillaries following a strong expiration. In fits of coughing, as occurs in whooping cough, the retinal capillaries have been reported to break.

In ethological terms, the expression of grief results from the superposition of two sets of motor patterns—the crying pattern together with the frontal-muscle inhibiting pattern. If this reaction occurs in a young child and is maintained over a period of years, both the physiology and structure of the face are altered when the child reaches adulthood. *The motor patterns of every impulse the child is called upon to suppress in his "socialization" requires that another motor pattern be used to inhibit it.* The inhibited or suppressed impulses, the forces behind unconscious repression, the defense mechanisms, the functions of the ego, and similar psychological entities are all brought about or maintained by motor patterns, which together with the motor patterns of the original impulses become superimposed and locked together in both the cortex and the body of the individual.

A knowledge of phylogenetically old behavior patterns in the human central nervous system is important, not only in unraveling the origin of emotional expression, but also in understanding the maturation of other forms of behavior. McGraw (52) and the pediatrician Peiper (55) pointed out that Ernst H. Haeckel's biogenetic law, "Ontogeny recapitulates phylogeny," is particularly applicable to the maturation of locomotion in the child. For the first 4 months of its life, the infant is capable of the fish–amphibian movements of the trunk (dog paddle), which support swimming. These are reflex in nature and need not be taught. Only after a few months more does the infant become capable of voluntary overhand-crawl-type swimming movements. Terrestrially, the infant and child is capable of amphibian–reptilian creeping and crawling types of locomotion, normally followed by erect, bipedal locomotion. The mammalian form of quadrupedal locomotion is also present in the human nervous system, and if a child is provided the opportunity to crawl over rough terrain at 10–14 months of age, he will reflexly snap up into a quadrupedal position and trot off like a dog. Figure 7D shows an 11½-month-old boy spontaneously walking quadrupedally on a concrete path.

Figure 7E shows a 14-month-old boy chasing after his mother on the concrete surface walk surrounding a swimming pool. He could both crawl and walk bipedally—the latter somewhat uncertainly. When he wished to move rapidly, however, he chose the quadrupedal gait, with which form of locomotion he could travel fastest. His mother informed me that, 3 weeks after this photograph was taken, he walked quite well bipedally and never again used the quadrupedal gait. When children this age are placed on smooth floors, walking on all fours is normally

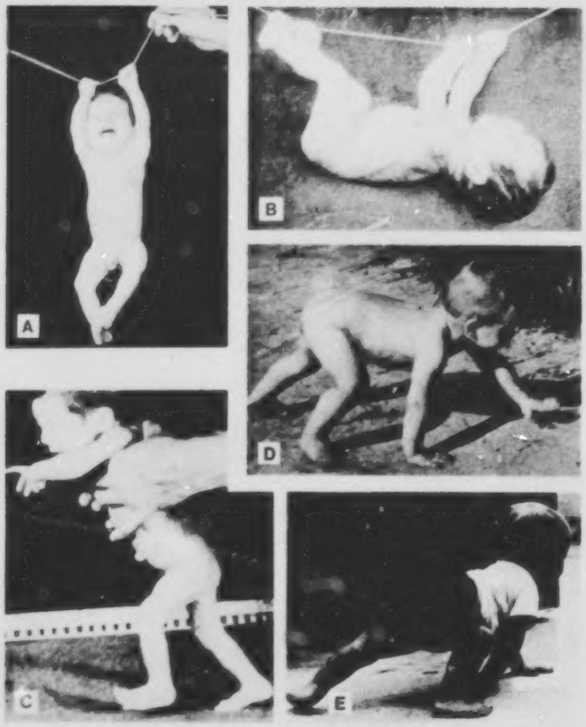

FIGURE 7 (A) Grasp reflex in a prematurely born, 4-week-old infant. (B) Arm and leg suspension with both palmar and plantar grasp reflexes. After Peiper (1963). (C) Walking movements in a newborn infant. After Peiper (1963). (D) Quadrupedal locomotion in an 11½-month-old boy. (E) Quadrupedal locomotion in a 14-month-old boy. Author's photographs.

not seen. A rough surface is instrumental in initiating the reflex quadrupedal position from which this form of locomotion emerges spontaneously. The primate patterns of clinging (Figures 7A and 7B) and bipedal locomotion (Figure 7C) are also present at birth.

All these demonstrations of innate, or inherited, patterns of vertebrate locomotion present in the human nervous system are an extension of Coghill's (8) (Figures 8A–8D) fundamental studies of the maturation of swimming and terrestrial locomotion in the tiger salamander (*Ambystoma tigrinum*). He demonstrated that the pattern of swimming

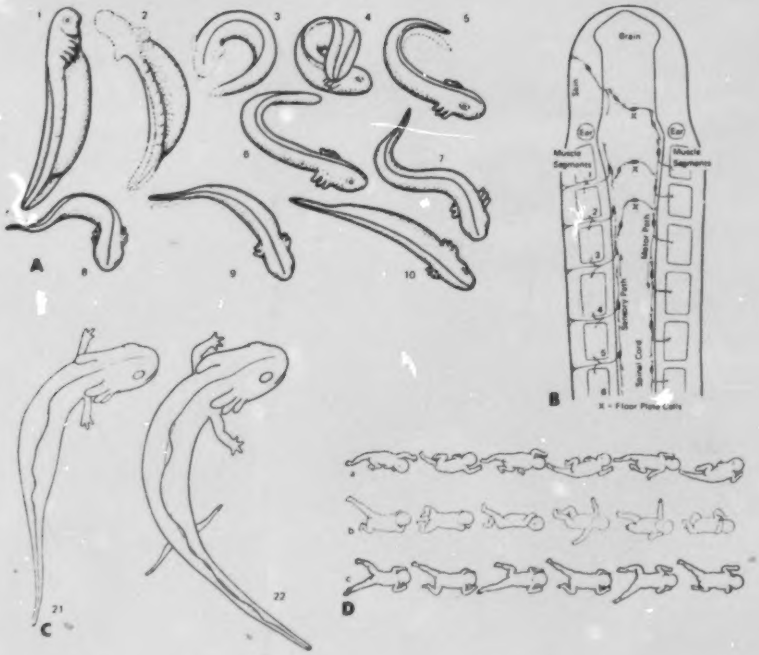

FIGURE 8 (A) Drawings from motion pictures of lateral flexure (1,2); coil movements (3,4), phases of the "S" reaction (5–10), which leads to swimming locomotion of the tiger salamander (*Ambystoma tigrinum*) embryo in the egg. (B) A section of the embryo showing the innervation of the muscle bundles and the floor plate neurons that make the "S" reaction possible. (C) Diagram of *A. punctatum*, illustrating the initial act of walking with the forelimbs alone, together with trunk flexure (21). Walking with all four limbs and trunk flexure, when the hind limbs have not yet developed reflex actions or knee flexion in response to local stimulation. With permission from Cambridge University Press. (D) Dog-paddle, trunk swimming in infant 1–4 months old (a). Uncoordinated movements in water, 4–6 months old (b). Voluntary, "overhand crawl" movements, 6-plus months old (c). Published with permission from Columbia University Press, 1943.

matures completely in 48 hours of development of the embryo within the egg (Figures 8A and 8B). These embryonic movements—lateral flexion, coiling, and the "S" reaction of reverse flexion, coincide with the maturation of the trunk musculature and successive phases of innervation of the mytomes and their cephalocauded innervation, together with floor-plate nerves that stimulate the reverse flexion. When the salamander develops legs and makes its way onto land, the leg movements are initially synchronized with the trunk swimming movements. The latter are gradually inhibited and quadrupedal, amphibian terrestrial locomotion emerges (Figure 8C). Terrestrial quadrupedal locomotion develops upon a foundation of swimming locomotion (Figure 8D).

This principle of the inhibition of earlier phases of vertebrate neural function in successive stages of vertebrate evolution is fundamental to the evolution of all higher vertebrate behaviors and functions, including the personality of man.

THE CONCEPT OF PERSONALITY

For almost 2,000 years in the Western World, it was considered that curiosity about nature, including human nature, was a sign of disobedience and sinful. This represented a tacit admission that the human psyche was, and should remain, an unfathomable mystery. Through psychology, the human personality again became a subject of interest, but we made little progress in understanding its development and nature until Freud, starting with various disturbed personalities and firmly dedicated to Darwin's evolutionary theory, returned man to his place in the animal kingdom. Although he employed a psychological methodology in unraveling it, he considered the development of personality from both phylogenetic and ontogenetic viewpoints.

In 1900 Freud (20) pointed out, "What we describe as our 'character' is based on the memory traces of our impressions; and moreover, on the impressions which have had the greatest effect on us—those of our earliest youth—precisely the ones which scarcely ever become conscious." In his "Three Essays on the Theory of Sexuality," Freud (21) adopted a more biological, developmental viewpoint in stating, "What we describe as a person's 'character' is built up to a considerable extent from the material of sexual excitations and is composed of instincts that have been fixed since childhood, of constructions achieved by means of sublimations and other constructions, *employed by effectively holding in check perverse impulses** which have been recognized as inutilizable. The multifariously perverse sexual disposition of childhood can accordingly be regarded as a source of a number of virtues, insofar as through reaction formation it stimulates their development."

Later, in 1908, Freud (22) pointed out that the character traits of orderliness, parsimony, and obstinacy were linked with the patient's concern about the anal region, developed during the period when this region came under voluntary control and cultural concern. He considered that other character traits might show a connection with the excitability and experiences of particular erotogenic zones. This, and subsequent papers, for the first time provided a link between biological events and psychological characteristics normally considered as motivation, attitude, and so forth.

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* Italics mine.

Freud postulated that the maturation of these innate patterns of behavior, together with their responses, inhibitions, correlated emotions, and experiences in the outside world, makes up the psychic structure that underlies human character or personality. If we translate our ethological concepts into psychoanalytic terminology, the infant's id functions ("all that is instinctive") can only be carried out by means of its fixed motor patterns, while the fate, or experience, of those motor patterns in the outside world (the individual way in which they are combined, integrated, or inhibited) makes up its ego structure ("all that is experiential"). That special portion of the ego experience and attitudes that results from the desire to maintain and prolong the close bond with the mother (the anthropologist Roheim's emphasis on the protracted infantilism of man) and to avoid confrontation with the powerful father, even to the point of reaction formation against its own id impulses, gives rise to superego formation. The chief function of the superego is the inhibition or limitation of satisfactions—that is, the suppression of behavior and the repression of associated thoughts and desires.

Freud considered that the doctrine of repression was the central finding of psychoanalysis. Together with behavioral suppressions, it is indeed the core of the process we refer to as the "socialization of the child." He later recognized that there can be no "cure" in psychoanalysis without affect, i.e., only if the process of free association leads to the expression of the old emotional feelings and motor patterns that are locked up in the psyche of the patient can he gain any insight into the unconscious motivations of his present-day behavior.

There appears to be a dynamic connection between the release of these emotional motor reactions and memory, for as analysis proceeds in this manner, earlier and earlier experiences, emotions, and memories emerge. The psychic ego is not separate from the id; it is built on the id and contains it. But, as I have pointed out, the inhibition or suppression of behavior can only be carried out by other motor patterns. Ultimately this suppression leads to a repression of the thoughts and ideas associated with such behavior. All of these mechanisms enter into the entities known psychologically as childhood amnesia and the unconscious. The child forgets these experiences, but the psychic structure, which has been developed, continues to function in slips of the tongue, every night in dreams, and in the motivations of one's daily behavior.

From the point of view of what is contained in its central nervous system, the infant is phylogenetically programmed at birth. As a result of utilizing these specific behavioral interactions with the mother, then other family members and peers, the infant's inherited emotional and neuromuscular responses are secondarily, or ontogenetically, programmed. This secondary programming of the neuromuscular system constitutes the psychic structure, or the character structure, of the child, which gives rise to the later personality of the adult.

It must be emphasized that all this structuring of these innate motor patterns are taking place simultaneously with the rapid growth processes occurring during infancy and childhood. Physiologic modifications, which occur during active growth, modify the nature of that growth. Behavioral modification, which involves autonomic, endocrine, and other physiologic changes, is equally capable of modifying growth processes, with the result that these early behaviors become anchored in the total somatic changes occurring during this period. There is no separate development of somatic and psychic structure, but a simultaneous integration that the term "character structure" is meant to designate.

Character structure is the result of all the behavior patterns that have become inhibited, recombined, and integrated in a characteristic way during early development of the infant and child. Although the oral, anal, and genital functions of psychoanalytic developmental theory play a preponderant role in this integration, they are not the only motor functions that enter into it. Erikson (16) recognized this in referring to the initial oral–respiratory–sensory stage of human development. This is followed by the muscular–anal stage, the locomotor–genital stage, and so on, through the eight ages of man leading to full adulthood and maturity. Just as Freud linked the psychological traits of orderliness, frugality, and obstinacy to the character structure, so Erikson links such character traits as basic trust, shame, doubt, initiative, guilt, a sense of incompetence, and confusion with the outcome of specific behavioral (motor) stages of early development. Cruelty, sadism, and neglect of animals are also character traits. The motor patterns of ethology are an integral part of human psychobiological development. Reich (58, 59) has explored and developed the biological relations between "muscular armor" and character structure, an exploration that subsequently developed into character analysis and then into vegetotherapy. The latter endeavors to deal directly with the disturbances of autonomic system functions that personality disturbances entail. Moreover, all of these infantile and childhood behavior patterns continue to be present in the nervous system of adults and may be spontaneously released in a type of psychoanalytic therapy termed "LSD analysis" (48).

Long before the current interest in body language, not only therapists but kinesiologists and orthopedic surgeons could agree that psychological elements contribute to every person's posture and movements. A recent edition of a textbook, *Movement Behavior and Motor Learning* (9), includes a chapter on personal equations in movement, dealing in essence with the influence of personality factors on motor performance. It is no longer sufficient, however, simply to recognize the psychological elements of posture and nonverbal communication.* The biologically lawful basis for these connections, which the neuromuscular basis of personality provides, requires incorporation into the general concept of human development and medicine.

The relations between ethology and psychoanalytic instinct theory have been more fully reviewed by Kortlandt (38) and Fletcher (18). The biological, instinctual basis of personality and its relation to medicine has previously been discussed in greater detail by the author (39, 41, 42).

It may appear a tremendous leap to go from ethology to psychoanalytic theory, early childhood development, and personality, but this is entirely the result of separate disciplinary terminologies. The common factor that bridges these two areas is the neuromuscular and autonomic nervous systems. The fixed motor patterns of ethology, which include the emotional neuromuscular patterns, represent the fundamental biological behavioral units of animals, including man. All of the early childhood experiences, the development of the ego and superego, are carried out by the primate, mammalian, and vertebrate motor patterns. The psychic structure and personality of man are formed around the phylogenetically old, instinctive, and emotional behaviors still present in the human nervous system—the fixed motor patterns of ethology (Figure 9).

ETHOLOGY AND OTHER DISCIPLINES

A volume edited by Fox (19) deals with the incidence and effects of a variety of abnormal behaviors in domestic animals. Marie-France Bouissou (7), working in France, reported at the Fourteenth Interna-

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* The Alexander technique (1, 2), a method of postural and respiratory therapy, not only corrects physical ailments, but also causes psychological problems to surface.

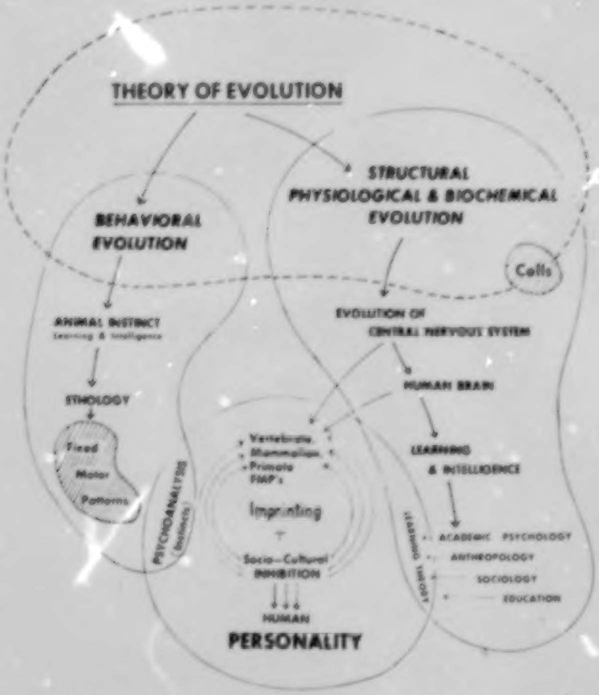
THEORY OF EVOLUTION
BEHAVIORAL EVOLUTION
STRUCTURAL PHYSIOLOGICAL & BIOCHEMICAL EVOLUTION
Cells
ANIMAL INSTINCT
Learning & Intelligence
ETHOLOGY
Fixed
Motor
Patterns
EVOLUTION OF CENTRAL NERVOUS SYSTEM
HUMAN BRAIN
Vertebrate
Mammalian
Primate
FMP's
Imprinting
+
Socio-Cultural
INHIBITION
HUMAN
PERSONALITY
PSYCHOANALYSIS
(Instincts)
LEARNING
& INTELLIGENCE
LEARNING THEORY
ACADEMIC PSYCHOLOGY
ANTHROPOLOGY
SOCIOLOGY
EDUCATION

FIGURE 9 Fragmentary development and thought regarding structural, physiological, biochemical, and behavioral evolution; the split in the connection between instinctive patterns of behavior, learning, and intelligence in man and other animals; the biological basis of personality founded on the sociocultural inhibition of vertebrate, mammalian, and primate motor patterns present in the human central nervous system.

tional Ethological Conference, held in Parma in 1975, on the effects of rearing conditions on the social behavior of cattle. Her findings show that cattle herds made up of cows that have been reared together from birth display more tolerance toward each other, even in competitive feeding situations, and tend to stay closer together in groups. On the other hand, cows that have been placed in herds 6 or 12 months after birth spend more time in agonistic behavior and less time feeding. The amount of time spent feeding is correlated with milk production. In this instance animal behavior research may not only contribute to the food supply, but also to the production of more contented cows.

Independent of ethology, the Bobaths (5,6) in London have been using innate motor patterns for several decades to restore the use of muscles that have lost their voluntary activity as the result of brain damage. Prechtl and Beintema (57) in Germany have developed a neurological examination of the newborn infant, which during the first 10 days of life can reveal abnormal signs, instead of waiting several years for the disturbances to manifest themselves in the behavior of the older child. It can also provide more exact knowledge of the relationship of obstetrical complications to neurological and behavioral disturbances later in life. Flehmig (17) in Hamburg has similarly been using a knowledge of human motor patterns, together with Bobath techniques (5,6) of physical therapy, to diagnose and correct brain damage at a very early age in infants and children (57). The late Albrecht Peiper (55), a renowned pediatrician in Leipzig, has long emphasized the importance of a knowledge of the neuromuscular system for the development and treatment of infants and children and the significance of ethology for future pediatric practice. Frederick Leboyer (44) has been utilizing the neuromuscular system to prevent, rectify, and treat the complications of childbirth in the newborn infant.

For the past 10 years, my ethology courses have included graduate students from the College of Education, and I have frequently lectured to Foundation of Education students. From the interest and enthusiasm they have displayed toward ethological concepts and principles, there can be little doubt that learning-theory psychology, which has so long supplied the bulk of the theoretical underpinning for educational methods, will also undergo some significant changes.

ETHOLOGY: THE BEHAVIORAL AND SOCIAL SCIENCES

Even as biochemistry has provided tremendous advances in biology and the medical and veterinary sciences, ethology will enter a similar role in the coming decades, not only in medicine and education, but also in the behavioral and social sciences. Is anthropology a biological or a social science? It is both, for, as we have seen, all social systems are fundamentally biological. Nevertheless, for decades anthropology has been separated into physical and cultural anthropology, with cultural and social considerations becoming more and more removed from biological principles and thought. With the rise of primatological research during the postwar years, and the demonstrations at the Japanese Monkey Center of subcultural, or early cultural, phenomena in the Japanese macaque, the sharp separation between cultural and physical anthropology is certain to diminish. The American Anthropology Association has already recognized this by creating a division of biophysical anthropology, which will include the recent studies of primate societies and behavior.

Until recently, anthropologists have paid little attention to the role of personality in human evolution and culture change. Nevertheless, Margaret Mead has for many decades emphasized personality, national character, and the mutual relations of cultural practices, child development, and social structure. LaBarre (43) and others have evaluated the national character of the people of particular nations. Hallowell (28,29) has, more than any other anthropologist, emphasized that the student of human evolution cannot disregard the evolution of the human psyche and its attributes—repression, identification, shame, guilt, and so forth—without which human social and cultural evolution could not have emerged. Indeed, if any individuals are judged not to possess these psychic characteristics, they are isolated in institutions and ostracized from society. The correlation and integration of man's psychic and physical evolution still remain to be accomplished.

Similarly, sociologists have almost entirely adhered to psychological learning theory and all but ignored psychoanalytic theory and the role of personality in their social studies. Sorokin (66), Parsons (54), and other sociologists, however, have begun to deal with personality or the vagaries of human nature in their researches and writings. Parsons (54) recognizes the significance of psychoanalytic theory for sociology, although he takes issue with certain of its aspects. Gerth and Mills (27) endeavor to understand how social structures shape the character of individual men and women. The biological model of man, whose behavior is based on certain mechanical or learned responses, dependent on his structural limitations, does not dovetail with the sociological model, which recognizes man's expectations and anticipations of others, in interpersonal actions. Consequently, they emphasize that man's psychic structure must be coupled with his biological structure if we are to understand and study the "roles that man plays" in various social institutions. Riesman (60) likewise stresses consideration of the changing character structure of man through the past centuries in interpreting the economic and political changes that have taken place in the course of history. In his emphasis on the evolution of human character, we return once again to biology.

Figure 9 provides a schematic indication of the fragmentation of thought and theory that followed the theory of evolution. Studies of comparative anatomy and physiology, even comparative biochemistry when established as a discipline, made great advances, but the comparative study of behavior, given a sound footing by Darwin's studies, did not prosper. Although given staunch support from time to time by the studies of Whitman (73,74), Wheeler (71,72), Plath (56), and Fulton (24), the taxonomic and comparative study of animal behavior did not develop as a productive discipline. Instead it was relegated to the quasi-scientific realm of animal instincts, not to be taken seriously by zoologists or biologists in general. Although anatomical and even experimental studies of the brain and central nervous system made progress, these approaches involved such an exceedingly complex organ system that little understanding of animal behavior was derived from them.

In the meantime, largely as a reaction to psychoanalytic instinct theory, academic psychology not only developed learning, reflex, and reinforcement theories, but also endeavored to do away with the concept of inherited patterns of behavior, or instincts, altogether. This endeavor was largely successful; generations of anthropologists, psychologists, sociologists, educators, and others grew up exclusively devoted to a learning-theory psychology, even though investigators such as Liddell (44a) and Masserman (49) demonstrated that psychodynamic factors were equally operative in the disturbances (neuroses) of man and animals.

In this fragmentation of thought, human personality became a psychological and vaguely defined characteristic, rather than a biological attribute, of man. Hopefully, the development of ethology, demonstrating the biological reality of fixed motor patterns together with the neuromuscular basis of psychoanalytic instinct theory and character structure, will render the biological basis of personality formation understandable.

All of this suggests that the behavioral and social sciences will ultimately adopt a biopsychosocial model of man, not only as they continue to assist medicine in its endeavors, but also for the development of their own disciplines. This development, together with the growth of ethology, indicates that animal behavioral research is certain to expand.

WHAT KIND OF REGULATION?

The continuing growth of animal behavior studies will undoubtedly make some kind of regulation necessary. I have underlined the development of the discipline and its potential contribution to the biological understanding of personality, because I know of no other way to emphasize the increasingly significant role that such animal research is certain to play in man's health and future social history. We know that with population increases, the social structures of animals change and evolve. In man, this has been accomplished through revolution and war, but we are beginning to question whether these methods must go on century after century. If the solution to man's social problems seems too lofty, idealistic, and a matter for future generations to consider, we are still left with the increase in degenerative diseases and illnesses that the population explosion is certain to provide and the problem of developing an equitable means of raising and exchanging adequate food supplies throughout the world.

With these developments we will have to carefully and fully consider the nature, effects, and implementation of any regulations regarding animal behavior research. I have before me a copy of the Cruelty to Animals Act of 1876, together with excerpts of sections that pertain to animal experimentation. On the whole, investigators in Great Britain are happy with the provisions and regulations of the act, although in some instances the inspectors, who are not trained, have an insufficient understanding of the animal research disciplines they oversee. I will outline a few of the questions that such regulations and their implementation pose.

1. Can we devise regulatory provisions that will not interfere with or impede animal behavior research and studies, as well as medical and veterinary research?
2. Should there be a licensing of experimenters, as provided for in the Cruelty to Animals Act of 1876 in Great Britain? How can we prevent this from becoming a licensing of experiments and an unwarranted interference with research?
3. How shall we define "pain" in animals? The deprivation of a rhesus monkey infant from its mother, as in the human child, is undoubtedly a painful experience. Yet these experiments by psychologists, ethologists, and physicians have unequivocally established that all primates, including the human infant and child, require regular periodic and daily physical and emotional contact with their mothers for normal health, growth, and social development. Recognition of these facts will become of increasing importance to mankind and must eventually enter into economic, political, and legislative decisions.
4. There are still relatively few people in our medical schools who are familiar with ethological research and its significance for human health and welfare. How will we attract ethologists to medical schools, and who will decide whether animal behavior studies represent significant contributions to knowledge?
5. Experiments dealing with social behavior and the stability of

social groups will require large field laboratories, such as in keeping rhesus monkeys in Cayo Santiago, Puerto Rico, or in feeding and observing macaque troops in their natural habitat at the Japanese Monkey Center. Should social behavior experiments come under the jurisdiction of legislative regulations designed for medical and veterinary laboratories?

6. What provision should be made to insure that the inspectors of such animal regulations have an adequate understanding of the nature of research in the various disciplines that require animals for investigation?

Cells, models, and systems theories cannot provide behavioral information about animals and man—information and knowledge about which we are only beginning to recognize our ignorance. Such animal behavior information will become more and more significant in the coming decades of the population explosion, and our still-incomplete understanding of the biological, sociological, economic, political, and national roots of aggression. It is certain that we cannot afford to hamper animal behavior research relevant to these problems, nor can we afford to deal superficially with the complex questions that the regulation of animal research will pose.

SUMMARY

1. During the decades following World War II, there has been a tremendous growth of ethology—a biological, evolutionary approach to the study of animal and human behavior.
2. These studies, dealing with the inherited and developmental aspects of a wide range of vertebrate behaviors, have stimulated extensive studies on the behavior and social structures of subhuman primates, as well as man, in the behavioral sciences and in education.
3. Ethology challenges the limited and erroneous views of academic psychology that there are no instincts in man and that man's behavior is the result of learning, conditioning, and reinforcement. This means that concepts and thinking in anthropology, sociology, and education, which have relied on learning theory in the past, will also have to undergo modification.
4. The biological fact that the human nervous system contains all the fixed motor patterns of its vertebrate, mammalian, and primate ancestry means that there will be a development of ethology in our medical schools akin to the development of biochemistry of the last three or four decades.

5. This, together with the development of ethology, is certain to correct our conception of the nature of human personality and revise our conception of the human socialization process.

6. The personality of man, so long studied psychologically and psychoanalytically, is actually a biological characteristic of man, resulting from the social and environmental modification of his neuromuscular system. This will mean that the separation of medicine into "hard-core" internal, obstetric, pediatric, and surgical medicine and "soft-core" psychiatric medicine will disappear.

7. Once we return to an evolutionary approach to animal and human behavior, animal behavioral research, which has increased tremendously in the last two decades, will continue to increase and become a vital part of our medical and veterinary schools.

8. This increase of animal research will undoubtedly make some kind of regulation necessary. Our concern must be that such regulation in no way retards animal behavior research and the valuable insights and understanding it is beginning to give us into the mother–infant relationship, the biological aspects of socialization, the role of social behavior in the growth and maturation of the infant and child, our understanding of the biological factors that enter into personality formation, and the significance of the latter for the stability of family life, communities, nations, and civilization.

9. There will undoubtedly be an increase of animal field stations and field laboratories in the coming decades. Animal regulations must provide that those who will be charged with inspecting such stations are trained to understand all aspects of contemporary animal research and its significance for the health and welfare of mankind.

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EVAN G. PATTISHALL, JR.

Animal Behavior: Relation to Illness and Disease

I should like to begin with a few observations on the use of animals in the study of behavior in illness and disease and then discuss the potential use of animals in order to teach basic human behavior.

Use of animals in biomedical and behavioral research has a long and productive history, and there are few diseases that have not been investigated using animals. Most of our knowledge, which has resulted in the understanding, control, and treatment of disease, can be directly traced to the careful and judicious use of various species of animals.

If animals had not been used in such research, it is safe to say that most of the diseases of 100 years ago would still be with us and perhaps running rampant through the populations of the world. It should also be mentioned that the same animal research that aided scientists to conquer many of the diseases of man has also aided man to conquer many diseases of animals. For example, nearly every drug that is useful to man is also useful to animals.

One of the major problems has been that, in spite of a tremendous investment in biomedical research during the past 30 years, proportionately very little of this has been spent on behavioral research, animal or human. The problem is that biology and biomedical science have too often been defined in the narrowest terms, with very little recognition that behavior is an integral part of biology and influences physiological, biochemical, and even anatomical factors, just as the same biological factors influence behavior. Dr. Kramer has illustrated this beautifully.

Practicing physicians have reminded us for decades that more than half of the patients they treat have a major behavioral emotional component related to their disease and that modern science must help them to learn more about emotional overlay and the interaction of behavior and disease (1).

One of the major deficiencies, I feel, of animal research in the past few decades is that most biomedical scientists have attempted to measure critical biochemical and physiological mechanisms and responses, paying very little or no attention to the psychological, social, or behavioral components of the animal being measured. It is as though the *in vivo* condition only involved the biochemical reaction, and behavioral factors, such as anxiety, fear, stress, fatigue, learning, and so forth, somehow existed in a world all their own and bore no relationship to the animal's biological functions. We now know that the behavioral factors do influence the biological factors, and, indeed, are an integral part of them.

Animal behavior investigators have likewise been deluded into thinking that the species of animal didn't matter much as long as they were dealing with the simplest life form that would be able to demonstrate a specific behavior. We now know that an animal's behavior, reaction, or learning is very much related to the specific species, to behavior genetic factors, to reactions to the testing equipment, to gender, to disease, and to the influence of specific biochemical and inherent behavioral mechanisms. For example, if one is studying learning mechanisms using an elevated maze, there are certain strains of rats that respond quite differently to heights; hence, their learning patterns are different under conditions of increased height, and the support or nonsupport of a certain learning theory can be directly influenced by this factor alone (2).

One thing we have learned is that you must know your animal. You must know its biology. You must know its genetic history. You must know its psychology, social habits, and so on, if you are going to put any faith in the results that you have obtained.

What may seem to work quite well in the simplest animal, or even in a single cell, cannot be assumed to work in the more complex animal or when multiple cells are combined. The old reductionist notion that we can separate complex tasks or complex biological mechanisms into their simplest forms by using the simplest species or cells alone is not necessarily valid.

In most behaviors we are dealing with multivarient mechanisms, each related to the other, so our testing and experimentation must also include the study of the behavioral and physiological mechanisms in their natural state or condition. The intact animal and its importance has been discussed in the previous papers.

One of the major proponents of the multivarient-of-natural-state point of view is Rene Dubos (3), who has commented that the study of the interplay between the component parts of the system is at least as important as a study of any or all of the isolated components. We now know that animals tested in their home-living or natural environments perform quite differently than those tested outside of their natural environments.

What does this have to do with the teaching of behavior? The point is that we have learned much from the study of the behavior of animals, and it has helped us with our study of human behavior. Through the intelligent and humane use of animals, we have been able to demonstrate certain behavioral mechanisms and we have been able to do the initial testing of these mechanisms. These results from animal studies can then be translated and applied to humans. Thus, we are indebted to animal studies that have provided the data to teach students about the generalizable nature of behavior.

My particular concern over the past 25 years has been the training of medical students. This has involved attempting to extract and synthesize a body of knowledge about basic human behavior from the biological, psychological, and social sciences and to translate this knowledge for those professionals concerned with the understanding and treatment of illness and the maintenance of health (4).

Let me share with you some of our major teaching objectives, and I think you will see how an understanding of animal behavior can be helpful to an understanding of human behavior. The teaching objectives of our particular core course in medical behavioral sciences at the Milton S. Hershey Medical Center are as follows:

1. Extending the scientific orientation of medicine into the field of human behavior at the individual, group, and collective levels of analysis;
2. teaching physicians how to effectively discriminate between levels of generalization in the patterning of human behavior, in other words, every person is in certain respects like all other people, like some of the people, and like no other person;
3. identifying psychological, sociocultural, and biological factors and their interactions as they relate to the disease process, the prevention of disease, and the enhancement of health;
4. developing skills of identifying behaviors associated with specific diseases and disease systems throughout medicine;

5. recognizing the ways in which the physician's own personality characteristics and feelings affect his interest in and reaction to various diseases, patients, and medical procedures; and, lastly,

6. recognizing the applicability of research methodology in observing, validating, interpreting, and predicting human behavior.

Consider the first objective: to extend the scientific orientation of medicine in the field of human behavior at the individual group and collective levels. In other words, how do you deal with behavior at the individual, group, and collective levels?

Let's look at the behavior of dominance and submissiveness. Jane Goodall has explored the social hierarchy relationships to be found in chimpanzees (5). An isolated chimpanzee swinging from a branch may behave as though he were content and self-sufficient as long as he is alone. But let a dominant chimpanzee approach, and he may immediately demonstrate submissive gestures toward the approaching male. As long as a dominant male is in his presence, he will continue to relate with submissive gestures.

By the careful study of individuals and groups, one can document the behavior of an individual alone versus the same individual in a group, and one can begin to appreciate how each chimpanzee or human learns his place and behaves accordingly in an extremely complex social organization. Similar analyses contrasting individual and group behavior can be demonstrated in almost any species.

The second objective—and they are not all of equal weight, the second, third, and fourth perhaps being more important—is teaching physicians how to effectively discriminate between levels of generalization in the patterns of human behavior; in other words, every person is in some respect like all other people, like some other people, and like no other person. This is a very important discrimination, I think, and it is one that seriously influences the practice of medicine.

I am sure you are all familiar with the physician who may have such generalized notions or stereotypes about human beings that he is apt to regard all persons as having the same characteristics. Examples would be: "All ghetto patients can't be trusted to take their medicine," "All school teachers are alike," "All fat people are undisciplined," or "All alcoholics are irresponsible."

One way to use animals to teach various levels of generalization is, for example, to have medical students practice observing primates, or other species, in group settings, taking particular note of nonverbal communication, the kind of thing that Dr. Kramer mentioned previously.

To the untrained observer, all primates tend to look alike, just like all Africans or all Indians or all Chinese tend to look alike to the person who has had no experience with such individuals. The task is to have the medical student look for physical and behavioral factors that will help tell one primate from the other. The student soon learns to identify those characteristics that are common to all primates, those social or cultural factors that are like some of the other primates, but not all, and finally those individually learned or evolved behavioral cues that are like one specific primate and no other.

The development of this kind of discrimination skill is especially important to physicians who often have a strong need to develop shortcuts in dealing with people and diseases by categorizing or pigeonholing both people and symptoms. Thus, the student can begin to appreciate that each species, including humans, has a wide range of individual variability and that he or she must learn to discriminate these variabilities and to integrate them into each patient-care situation. I am concerned with the dehumanization patterns that often develop with humans, as well as with animals.

The third teaching objective, which deals with the interaction of the psychological, sociocultural, and biological factors as they relate to the disease process, can best be taught through the use of the animal model. Such topics as the impact of stress on the biological mechanisms as in Selye's (6) or Wolf and Goodell's (7) studies, or the influence of maternal deprivation on behavioral or physiological development, such as Harry Harlow's infant love studies (8,9), can be most clearly and convincingly demonstrated through the use of animal studies. To try to use human beings rather than animals, to demonstrate or study such very important phenomena, would be extremely difficult, if not impossible.

I am well aware that such studies of stress and maternal deprivation are vulnerable to the criticism of the abuse of animals, and I would insist that the highest levels of humane treatment be maintained, but the problem is how can we study such factors as stress, deprivation, and aggression without submitting animals to some kind of stress, deprivation, or aggression?

The dilemma is: What is it worth to society to obtain certain information and what is the cost to society if we do not obtain such information? I submit that our ability to use the animal model has allowed behavioral scientists and others to gather data and gain an understanding of some very basic behavioral and physiological mechanisms that I feel are necessary to the survival of the human race. The fact that we can use these animal models in our teaching of basic human behavior is an important additional asset that cannot be replaced completely by human models, by computer simulation, or statistical manipulation, even though those are helpful tools.

Another important teaching objective that can often utilize animal studies in preference to human studies is that of identifying behaviors associated with specific disease and disease systems, and this includes stress, which I have already cited above (6,7). One of the most easily demonstrated diseases is the duodenal ulcer, but there are many other disorders that have already been mentioned and that can be caused by certain behaviors or that can lead to ulcerations or changes in specific behaviors.

As another example, I might cite some animal research that has been conducted along this line in our own Department of Behavioral Science at Hershey. The influence of certain biological alterations, such as ablation of certain areas of the brain, specifically the amygdaloid, can produce behavioral changes or deficits that are recognizable and measurable. Such behavioral changes can then be used by physicians to determine the exact location of a particular brain damage or lesion area.

There is no way that this kind of brain behavior information can be obtained through the use of human beings. I have some other examples in terms of critical periods, which Dr. Kramer already mentioned. I won't go into them, except to say that, again, working with animals, we are demonstrating that you don't necessarily overcome an initial biological brain or neurological deficit that occurs at an early age; the thought being at one time that damage at an early age can be overcome and that eventually one catches up versus having the brain damage or neurological damage continue to adulthood. The facts appear to be that you do not ever catch up (10).

Therefore, there appear to be critical periods, which, if interrupted by trauma, would result in severely affected subsequent behavior, extended into adulthood. I think one of the most exciting developments today in this area of disease and illness, as related to physiological and neurological and learning variables, is the ability of specific physiologic organs or mechanisms to learn specific responses through selected reinforcement. This is the autonomic nervous system research of Neal Miller and others (11), and I think it is probably one of the most exciting as far as the future is concerned.

Let me move on to the fifth objective, recognizing ways in which a physician's own characteristics and feelings affect his reactions. This doesn't appear to be relevant for animal behavior, and yet students can quickly recall the different feelings they have had toward certain animals based on former learning experiences and attitudes—snakes, for example, or horses, alligators, dogs, mice, and so forth. I am sure that each of us has different reactions to each of them, and working with animals is a good way to experience this.

It is easy for students to deny that they have different feelings towards different patients or diseases, because they have accepted the belief (or think they have accepted it) that they are supposed to treat each person equally and reject no one. But when it comes to animals, they can recall definite preferences, feelings, and attitudes. As they look at their own feelings towards certain animals and compare them with humans, they can begin to see that they also have less-recognizable, but real, feelings towards certain people as well.

I would carry this perhaps a step further. I would say that if you do not have compassion for animals, I doubt that you have compassion for people. I don't know whether you can use this as a selective device for admission to medical school or not, but I would be willing to suggest that it might have some merit.

The sixth objective, recognizing applicability of research methodology in observing, validating, interpreting, and predicting behavior, is one of the more important teaching objectives related to animal behavior. Even though I feel that methodological problems are the major issues of the future in terms of use of animals, medical students can grasp quickly that the research methodology used in studying behavior in animals is very closely related to the study of human behavior.

Equally important, they soon learn that it would be impossible to study certain critical behavioral mechanisms if they could not rely on the use of animals for such research. For example, in studying maternal deprivation, we are able to show them the present state of knowledge, using the very few human studies that are available, and to point out the large gaps that must be filled in if we are going to understand how maternal deprivation or interpersonal stimulation affects human growth and development.

They can then appreciate how important it is to have the animal model, which can be used to stimulate human mechanisms, as in Harlow's studies (8) already mentioned, and to gain valuable information that would be virtually impossible to secure otherwise.

This is a good example of how animal studies have been used to verify earlier untested observations of humans. For instance, Rene Spitz in 1945 (12) reported that children reared in deprived, motherless, institutional environments with good medical and nutritional care still evidenced severe intellectual and growth deficits, compared with children reared in similar, but more interpersonally enriched, environments.

This led to the untested presumption that early environmental restriction impairs intellectual development, while early environmental enrichment enhances intellectual development. While this could not be tested through human manipulation, subsequent animal studies have clearly demonstrated that animals reared in enriched environments were superior in learning tasks and superior in using other developmental criteria than animals raised in less-enriched environments. This has enormous implications for human education and human development.

In summary, it would be impossible to manipulate human subjects or to carry out many of the most important studies over long periods of time that are needed in understanding both acute and chronic diseases. Animals are especially important in studying chronic diseases because most animals have a shorter life span. Specific examples would be the use of animal models for studying such diseases as duodenal ulcer, hypertension, coronary artery disease, cancer, strokes, emphysema—all of the long-term chronic diseases that, even if we could use human subjects, would be impossible to replicate in human beings in less than over a 10- to 30-year span of time, which is the time it takes to develop some of the most important chronic diseases in humans today.

It is especially crucial that we utilize the animal model in the study of the interaction of behavior with illness and disease. Furthermore, we must make greater utilization of the animal model to teach an understanding of human behavior.

In the past, we have taught medical students basic physiologic processes by observing animals. Now we must recognize that behavior is a basic biologic phenomenon and that we can also illustrate the behavioral growth and development and the range of variability through the use of animal studies.

I can think of no better way than to teach medical students, at the earliest stages of their training, to be compassionate, to solve medical and human behavioral problems, to teach ethics applied to human beings and to animals, and, most important, to teach the student about man's place in nature.

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DISCUSSION

PFEIFFER: I have prepared a few remarks in the nature of a discussant rather than as a questioner. I think we have gotten some idea of the perspective and potential ethology has to offer its sister disciplines. Social science has been perhaps most resistant to this zoological perspective. In my own field, anthropology, it was not until the early sixties, through the work of Washburn and DeVore, that the relevance for the study of man of animal behavior studies began to be taken seriously.

Still, as one reviewer has pointed out, much of this work was and continues to be kite-flying, a provocative displaying of possible and occasionally probable connections between biology and behavior. The idea that man has limits, be they biological or otherwise, has not been wholeheartedly received by discipline, which teaches that man is infinitely perfectable and perfectly infinite.

Anthropology aside, it should be mentioned that the first publication entitled *Human Ethology*, by a student of Tinbergen, appeared in 1969. Not only is the discipline new, but its practitioners come from many fields and focus on disparate problems.

Issues of the *Human Ethology Newsletter*, published by a group in England, contain ongoing attempts to define what human ethology is all about. Since it is, if anything, young, it is not surprising that medical schools, pragmatic institutions that they are, have not fallen over themselves in the rush to embrace it.

There is, however, a Veterans' Administration hospital in California that is planning to have its psychiatric residents devote their first 6 months to studying monkey behavior. The idea is to sensitize them to a nonverbal behavior, as Dr. Pattishall suggested, and to teach them behavior-observation techniques.

These behavior-observation recording techniques will probably prove to be one of ethology's most significant contributions to health sciences. It is no news to clinicians that psychiatric pigeonholes don't pigeonhole people very well. The diversity, for example, among people labeled schizophrenic, or even simple schizophrenic, is staggering.

Ethology emphasizes watching what people do and sampling what they do, operationally defined behaviors or sequences of behaviors, such that another observer can know what was observed. A group of us from the Rutgers Medical School did such a sampling of behavior among two different tiers of the same prison. Although we found the tiers to be very different, there was a lot more aggression on one of the tiers, and the prison authority's judgment agreed with our findings; the psychiatric profiles of the tiers were similar. If diagnostic categories don't tell us anything about behavior, what good are they?

Turning to animal behavior studies and the subject of this conference, I would like to consider some of the challenging questions posed by Dr. Kramer at the end of his paper. First, how shall we define pain in animals?

I suggest that the difficulty of this procedure has been exaggerated. Chimpanzees, rabbits, and other animals scream in the same language that we do, for example. With other species, judgment might be a bit more tricky. The dog, however, yelps and, for more subtle cues to his pain, one need only consult J. D. Scott's classic ethological work on the social behavior of the dog. I might add that his work is based on quantitative analysis of the context of behaviors observed.

We know that a group-living animal, as shown by field study, is in pain when it is isolated from its kind. Harlow received a lot of press attention, but one has only to observe that the most ubiquitous social bond in the mammalian kingdom is the mother–infant bond, and its importance for normal growth and development is abundantly clear.

Earl Count noted this in the early fifties. Jane Goodall made the case as strongly as Harlow for the mother–infant bond when she observed chimpanzee infants orphaned by contact with a human-induced polio epidemic. She was able, moreover, to document how other group members, most interestingly blood relatives, helped or failed to help these infants.

This example also illustrates how much responsibility we assume when we undertake to study animals, even with relatively minimal intervention. I think that Harlow's point could have been made by simple observation. Michael Chance, the British ethologist from Birmingham, is fond of a point that bears paraphrasing here: Astronomy, the oldest and one of the most rigorous sciences, has never manipulated a variable. Rather the years of recorded movements, the behavior of the stars, comprise this data. Watching the stars may lack the drama of prolonged social deprivation *a la* Harlow, but the data obtained are just as solid, the analysis is predictive, and the absence of subject sacrifice is a redeeming virtue.

Man is indisputably the best model for man, although I am not suggesting that we perpetrate our studies on each other, though I know many researchers who actually do so or are frankly eager to do so. Maybe there would not be so much of a perceived need to experiment with nonhumans if the experiments the physician conducts daily under the guise of practice were more widely communicated.

Think of what we would know of human problems if each doctor's results had been plugged into a central data bank over the last half century and subjected to analysis. Computer technology now available makes it possible to coordinate and disseminate information in unprecedented ways.

Think of the data that has been lost by students of human behavior, as in the case of psychiatric pigeonholes that don't pigeonhole because we had a lousy methodology.

Dr. Kramer mentioned the concept of a field laboratory, which I am involved in. A group of us from Rutgers University has established a chimpanzee breeding facility at Lion Country Safari in Florida. Such projects keep the animals more humanely and recycle animals that would normally be sacrificed. The animals are available for a wider range of investigation, particularly behavioral. It takes the pressure off the wild, and it is cheaper by some 1,000 percent.

KRAMER: I would like to comment about one remark that Dr. Pfeiffer made. He indicated, for example, that we could make Harlow's point by simple observation, that is, the importance and significance of the mother for the development and the health of the human infant.

We have observed many of these things, and yet in our medical schools today we still have a fragmentation, to a considerable extent, of medicine between what is known as "hard-core" internal, clinical, pediatric, obstetric, surgical medicine and "soft-core" psychiatric, psychoanalytic, psychodynamic medicine, which has been looked at for so long as sort of on the fringes of medicine.

These attitudes in our thinking have persisted for decades, in spite of all the work that has gone on psychodynamically and psychoanalytically. Yet, Harlow's experiment was a very important psychological contribution, because it made us aware that we do have an evolutionary background and that the things that are important for primates are in our nervous systems. It demonstrated that very nicely and in such a way that many opponents of psychoanalytic theory criticized, namely, that this was all something simply going on in the analytic room of a psychoanalyst and was not really scientific because it was conducted on this psychic-room method.

I think that this is very easy, as, for example, when Columbus demonstrated how you stand an egg on its end, and everybody tried and failed. Columbus supposedly, the story goes, simply cracked the end of an egg and put it up and stood it on end. After it is done and we have got these experiments, we all say, "Oh, of course. Well, we knew that all the time." And yet it takes contributions from many fields to make us aware of the fact that a certain concept, way of thinking, or methodology is significant, and we are still engaged in that process.

PRATT: I also would like to make a comment as a psychiatrist. It is possible there has been this cleavage that Dr. Kramer refers to between psychiatry and other forms of medicine. Certainly one remembers it in one's own medical school experience.

However, I do think that there is a great body of literature available in the field of psychiatry, especially child psychiatry, which is certainly most instructive. Many of the things that you mentioned have been demonstrated over and over again. It seems extraordinary to me that, in a sense, you are suggesting medical schools turn their back on this kind of material, which is available, and instead look to the kinds of experiments that are identified by Dr. Harlow's work.

It is hard to see how medical students can learn compassion from the kinds of experiments that Dr. Harlow is associated with. I would like to ask a question of Dr. Kramer, because I seriously find it difficult to know what is meant by ethology. Dr. Harlow's experiments tremendously distort the environment. It is impossible to imagine that the monkeys that he has worked with bear much relation to primates living in their natural environment.

Am I to understand, however, that ethology can include such distortion of the environment as Dr. Harlow has been associated with, or do you say that isn't ethology—that is animal behavior work, psychological work—and do you draw a line of differentiation there?

KRAMER: Dr. Harlow's work was conducted in the Psychology Department of the University of Wisconsin. He had just begun that work when I was there a number of years ago, and you are perfectly right in indicating that slowly it will become more and more difficult to differentiate between psychology and ethology.

Yet there are certain basic concepts that underly ethology that have not been present in psychological work.

I might emphasize those concepts by saying it was very significant for physiology, anatomy, and biochemistry when we long ago realized the fundamental unit that the cell represented for living things. It doesn't matter that today we are going beyond the cell. We are getting to subcell types of living and experimentation. But that concept of a unit has been extremely significant for the development of all sciences.

The concept of the fixed motor pattern, which is characteristic and fundamental to ethology, is not a part of psychology. I want to emphasize the significance, and I haven't done it sufficiently, of the fixed motor pattern.

No animal, including man, can move a single muscle. It is impossible to do. All that man can do is make movements, that is, carry out motor patterns. The motor pattern is the behavioral unit. It is the unit of all animal behavior, and, whatever the social system does in the way of socialization, it acts on these motor units lawfully within the nervous system. If you asked me point blank, what is the significant difference, I would say it is the biological recognition that there is an entity of behavior called the fixed motor pattern, a genetically determined, neuromuscular coordination that underlies all vertebrate animal behavior.

FREE: Could the ethologists present give us some justification for the inducing of stress through the permanent caging of dogs not used for psychological studies?

KRAMER: I know the question is addressed to me, since I am the ethologist here, but I am not aware of the experiments you are talking about in stressing dogs. I really cannot say anything on the experiments you are asking about.

Harlow's experiments, which I frankly admit are stressful, are certainly stressful to the rhesus monkey infant who is separated from its mother. I would agree also with Dr. Pfeiffer that we can know pain in animals, although we would have to revise our concepts of dealing with it and anesthetizing animals.

I only wanted to bring out that there are many considerations that we must look at. It is painful, for example, when Harlow's rhesus monkey infants go into a corner and sit there quietly. They are very quiet and not screaming out, but they are stressed and in dire difficulty. There is no question about that.

All I was trying to indicate is that many of these experiments, when compared with the human concerns about health and welfare, have to be evaluated. I am not ready at this point to make any judgment as to what is stressful or what kind of stressful experiments should not be done.

I would agree with Dr. Bustad that sensitivity is an important factor. As for students, physicians, and people doing animal research, these are all things we are concerned with. Medical and veterinary schools have been endeavoring in their selection to be concerned. As was pointed out, intelligence is not the only guide. Especially in human and veterinary medicine, a concern with animals and the welfare of patients is vitally important.

FREE: Putting aside the debate on Dr. Harlow and that situation, I am concerned with the inducing of stress for no reason, not in the experiments. You might say it is found in the storage of dogs over many, many years.

It has nothing to do with experiments in that sense of the word. It has long been a bone of contention, since the Animal Welfare Act was first passed, that large numbers of the scientific community answered negatively to the Department of Agriculture's statement in the *Federal Register*.

What do you feel, Dr. Pattishall, about the stress induced because dogs are just held in what you might say cold storage?

PATTISHALL: I find it a little difficult to respond because of my own lack of experience, having been privileged to be at two institutions, particularly now at Hershey, where I feel we have ideal animal conditions, developed by Dr. Harrell and Dr. Lang, where you will not find this kind of stress. So I want to be sure that everyone does not, or the implication is not, that all or most dogs kept at medical facilities, whether they be behavioral research or physiological research, are kept under conditions of extreme stress. We do have a few, I think, excellent models in operation today.

This brings forth the question of methodology. I think if we are going to stress animals to study the effects of stress, it seems to me that we should simulate the human condition as much as possible. Now, obviously Selye's studies, where he tied the rats down and put them in cold storage and so forth, is not a human simulation at all. It is a condition that I assume does not exist, at least in many medical schools. We don't treat our students that way, but nevertheless I think we have to look for the methodology of similar or existing conditions of stress in the animal kingdom as exist in the human kingdom and thereby study this. In cases where we must generate stress in order to understand the mechanisms, then it would seem to me that we can profit by or have the results of our research be much more directly related to human endeavors.

FREE: My main thrust was simply that these are animals not kept for stress studies. Stress becomes incidental and is not the purpose of having the animal there. The stress develops, and distress, because there is no other place to keep them or an unwillingness to provide different types of quarters. That is all I was asking. It is very simple.

BALAZS: I am not sure that I understood. Caging animals individually for an extended period itself represents stress, if one defines stress as increased production of corticosteroids and its physiological consequences.

I think we keep research animals in this country better than any other animals. There is no other practical way but to keep dogs in cages. We cannot have a bedroom, bathroom, living room, and so on.

CORNELIUS: The reason I have had some introduction to this myself is that we have a major program at the University of Florida. We are trying to find out what are the most humane environmental requirements for animals. Major chambers are set up where animals are put in what we hope are ideal environmental conditions. When one makes the statement that animals in cages are stressed, it is a subjective decision on what the stress of the animal is.

When one starts looking at the medical implications of what stress may be in the animal, it is very difficult to find hard-core data outside the subjective impression that it is wonderful for animals to have total freedom in moving around.

I know of very little research, if any, that really specifically says that animals, under certain types of caging conditions, are stressed. I know this becomes a subjective thing of what a person feels an animal should be under. I think that it is almost a situation where you would like to have every experimental animal much like your own pet at home.

ANCHEL: As to the question of why animals are kept under stress when they are not under experiment, part of the problem is that the fact that they *are* under stress has not been admitted by scientists. I believe that the way this has been investigated, measurement of urinary ketosteroids, is not at all suitable. I think it has been pointed out in this symposium that you have to select the proper models. If you were trying to select the proper model for studying whether a man is under stress when he is in jail or in solitary confinement, you would select just such a model, namely an animal caged and in isolation. Conversely, man can serve as a model for the animal. A man in solitary confinement is under stress, and he doesn't have to be tested any further to know that. Extrapolating back from man, you will understand that the animal, too, under these conditions, is under stress. What has been proposed is not "bedroom and bath" for the dog, but simply enough space to walk about in and a modicum of socialization.

GREENSTEIN: We are preparing what is called the Laboratory Animal Data Bank, which is a method of transmitting collected information to the scientific community. It relates to this particular question in that you take the information about environmental conditions at one institution and transmit it to another institution that is trying to get information stored in a computer system. You will then be able to examine your own environmental situation in light of the environmental situations throughout the country.

If you evaluate and conclude, "We are getting bad results with the same kind of animals," you may then be able to say, "Possibly some of my environmental situations, such as keeping a dog in a cage, not exercising him, are at fault." This will be a method of evaluating the environmental situation. I offer it as evidence that the scientific community is concerned and that they are trying to do something about the situation by examining and policing themselves. Although we are slow at times, we do want to look at data rather than opinion, and I think this is one method of doing it.

LEO K. BUSTAD,
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Animal Models*

INTRODUCTION

During the past two decades, animal models have become increasingly important as means of studying disease processes and entities in people. Although the principal objective of studies on animal models has been the alleviation of diseases in people, it is important to point out that studying the disease process will benefit animals as well.

We recognize that much useful information has been obtained by inducing in animals disease conditions that hopefully mimic naturally occurring diseases in people. Because of this method, many diseases caused by infections, toxic agents, and malnutrition have been elucidated, treated, prevented, and/or otherwise controlled. But the relationship between causative agent and pathophysiological process is less simple and direct with many of our major diseases; rather, they seem to involve complex interrelationships. What is needed, we think, is a concerted effort to obtain more naturally occurring animal models of spontaneous disease. Such models will provide opportunities to study the disease entity from its inception in the laboratory, as well as in its natural environment.

With this in mind, we collected references on animal models from a variety of sources with the intention of updating the helpful reviews of

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Cornelius (16), Leader and Leader (57), Doyle *et al.* (22), Jones (47), and others. When we exceeded 6,000 references for induced and naturally occurring animal models, we decided that updating only the naturally occurring diseases would be more within our physical capabilities and also would reflect our principal current interest (see Appendix).*

Even with these limitations, in view of the magnitude of the possibilities, it was frustrating to pick and choose the areas to discuss. Sometimes the line of demarcation between induced and naturally occurring disease becomes quite obscure. Because of our experiences, current interests, activities, and, most importantly, the significance of the particular models, we have chosen to discuss genetic and congenital diseases, chronic degenerative diseases, and cancer. Because of the relative paucity of data on genetic disease and because of the activities of two of the authors, Hegreberg and Padgett, simple Mendelian genetic disorders are given special emphasis. Cancer is a special interest of Bustad, the senior author.

GENETIC DISORDERS AND CONGENITAL MALFORMATIONS

Studies of genetic disorders are needed to define specific hereditary traits in order to differentiate and diagnose neonatal diseases, to detect carriers of recessive genes and to determine what birth defects can be attributed to known genetic factors and to understand the biochemical mechanisms involved in the disease process. The results would have direct application to animal breeding and to clinical situations in human and animal medicine, where they affect the advice the clinician gives his client. Such studies in animals can be prospective, but in people they are almost always retrospective and, therefore, not totally accurate. Additionally, defined genetic disorders in animals provide a matrix for uncovering mechanisms and developing rational therapy for homologous human disorders and for solving fundamental problems, including mechanisms of aging and host resistance or susceptibility to acquired diseases. But valid comparisons of animal diseases to human disorders are contingent on a lucid characterization of the disease in both people and animals.

Research workers spend much of their professional lives establishing validity in comparisons, eliminating superfluous variables in experimental studies, and establishing reproducible conditions. Genetic studies, especially those involving simple Mendelian traits, offer the distinct advantage of simplicity. The clinical and biochemical characteristics of most simple Mendelian disorders, especially metabolic derangements, are such that a novice can readily recognize affected and nonaffected controls. Simple Mendelian traits provide a disorder with a single primary variable by which one can converge on a specific metabolic change and, subsequently, define the effect of that metabolic derangement, be it enzyme or structural protein, on the entire host. Furthermore, working with genetic disorders allows study of a natural, reproducible situation. Induced disorders often introduce exogenous factors that create conditions that are sometimes difficult to relate to the original, spontaneous animal or human disorder (77).

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* Due to the length of the Appendix, it is available as a separate publication, free of charge, from the Institute of Laboratory Animal Resources, 2101 Constitution Avenue, Washington, D.C. 20418.

Studies of spontaneous animal diseases, including genetic disorders, have been neglected. In substantiation, one has only to compare the total number of simple Mendelian traits in people (approximately 780) (67) to the simple Mendelian traits in dogs (approximately 50) (16) or the more thoroughly studied mouse (approximately 306) (67) to note a gross numerical deficiency of proper, animal genetic diseases studied and recorded. One suggested explanation for lack of attention to this important area is that a single genetic defect usually has a small impact on the overall disease problems of a given species. However, when we confront such major diseases as dwarfism in Hereford cattle and Alaskan malamutes and progressive retinal atrophy in poodles, we realize that genetic diseases represent, cumulatively, a problem of major concern.

Undeniably, much has been accomplished in defining valuable animal genetic models of human disease in the past few decades. Established models include globoid leukodystrophy in the dog (29); muscular dystrophy in the chicken (2), mouse (68), and hamster (45); GM_1 gangliosidosis in the cat (3); cyclic neutropenia (60) and the Ehlers–Danlos syndrome in the dog (42); the Chediak–Higashi syndrome (79) in cattle (80), mink (78), mice (61), and whales (99); the Dubin–Johnson syndrome in sheep (17); the Klinefelter's syndrome in the cat (101); porphyria in cattle, swine, and cats (31); various types of hemophilias in dogs (10,37), horses, and swine (88); spontaneous hypertension in several strains of rats (18,75); and "wobbler" mouse, a lower motoneuron disease (8).

For future consideration, rational approaches in the treatment of both acquired and genetic disorders of people and animals should be developed from basic information obtained in studying specific animal metabolic derangements. The mechanisms of abiotrophies should be continually sought. Increasing knowledge of superovulation, semen preservation, and egg-transfer techniques in some species will expedite genetic studies and animal production.

Hopefully, a more refined and complete knowledge of genetic disorders will lead to a better understanding of nutritional and infectious processes. In this regard, one of the more interesting areas to explore is the genetic aspects of susceptibility and resistance to infectious diseases. Most of us know at least one person or animal that never seems to get sick and others that always seem to get sick; both of these traits probably are genetically determined, in the light of evidence available from studies in animals. Webster in 1937 (104) showed that resistance to a specific bacterium and resistance to a specific virus were single gene traits and that they were nonallelic. He further demonstrated that resistance to two unrelated viruses was nonallelic in mice. Gowen (36), in an excellent series of reports on chickens, showed that resistance or susceptibility to one bacterial agent did not indicate resistance or susceptibility to another unless the organisms were closely related. Several other investigators have shown that susceptibility to one virus and resistance to another are nonallelic, single-gene traits. Sabin (90) and Goodman and Koprowski (35) demonstrated that the concentration of virus was consistently lower in resistant strains of mice than in susceptible animals, as did Kantoch *et al.* (48) using mouse hepatitis virus. The important point here is that susceptibility and resistance to a large number of bacterial and viral agents are genetically determined as simple Mendelian traits. Exploration of the factors involved is of great significance if we are to reach our goals of long, productive lives and good health for both people and animals.

Several inherited disorders of people and animals are accompanied by decreased resistance to disease. The Chediak–Higashi syndrome has been studied in people, cattle, mink, mice, cats, and killer whales. An increased susceptibility to infectious diseases accompanies this disorder. There is a functional reduction in the mobility and in the bacteriocidal and degranulating ability of neutrophils. Lysosomal enlargement is the hallmark of this disease and reflects an apparent fault in lysosomal membranes. A combined immunodeficiency of horses, which mimics severe combined immunodeficiency, the so-called Swiss-type agammaglobulinemia of people, has been recently identified (65,68). This disorder, observed in young Arabian foals, is accompanied by severe deficiency of both B- and T-lymphocytes, inability to synthesize immunoglobulins, and increased susceptibility to viral, bacterial, and fungal infections. These animal models represent the growing list of defects in host resistance that will be important in defining the underlying mechanisms that differentiate health and disease.

Comparative studies of spontaneously occurring congenital malformations are limited (58,83). The causes of many human birth defects are unknown; statistics concerning the hereditary versus nonhereditary causes are unavailable. Studies of animals should prove rewarding, for considerable information is available on their spontaneous birth defects. Veritable epidemics of congenital malformations have been observed in domestic animals, and many have been defined. Causes have been traced to genetic and environmental factors, viral infections, intoxications, and malnutrition (71).

In these areas, the future's needs are great. Sufficient numbers of undefined, animal genetic disorders and congenital malformations exist to engage multitudes of investigators for many years. Specifically, we need to define both chromosomal anomalies and the underlying causes of developmental defects such as cleft palate, cardiovascular anomalies, and malformations of limbs. In this regard, high priority should be given to carrier detection and early, *in utero* identification by amniocentesis. In concluding this section, we must point out that genetic birth defect studies take time, materials, and space (4), more so the larger the animals. The high cost of such research is probably the most valid reason for the slow advancement of knowledge about these diseases.

CHRONIC DEGENERATIVE DISEASES

Of the chronic degenerative diseases, cardiovascular disease quite deservedly has been given the most attention. More consideration should be given to some of the other degenerative diseases, including those involving autoimmunity, connective tissue, muscles, joints, and the central nervous system. Such chronic degenerative diseases have few defined spontaneous models, perhaps because we have not directed ourselves to these areas. However, it is clear that to achieve major progress, we must give these problems a high priority and search more diligently for animal models for them.

Although multiple factors may be involved in events leading to similar chronic, clinical, and pathologic states, a better understanding of the basic mechanisms of chronic degenerative disease may be gained by studying the condition early in the course of the disease to determine the first, and presumably the primary, influences. Careful examination of single hereditary, metabolic or nutritional factors may provide a basis for future studies. The most promising approach appears to be to find natural diseases in animals and develop them as models.

Cardiovascular Disease

Several chronic degenerative disorders present formidable and urgent challenges to the biomedical professions. The most impressive statistic in this regard is that coronary heart disease affects one in five North American men by the age of 60 years (96). Coronary heart disease is currently the chief cause of death in the United States. Hypertension, which is estimated to affect 10 percent of the adult human population (106), is one of the most cogent progenitors of cardiovascular disease, especially atherosclerosis and myocardial failure.

Recent studies indicate that people may manifest significant elevation of blood pressure at relatively early ages; it has been speculated that familial factors play an important role in the genesis of high blood pressure (108). Several strains of rats with inherited hypertension have been identified and characterized to serve as naturally occurring models for study of hypertension. The spontaneously hypertensive rats reported by Okamoto (75) and Dahl *et al.* (18) provide not only a highly reproducible disorder, but also their blood pressure is markedly elevated at any early age. In advanced stages, this condition is accompanied by a high incidence of hypertensive cardiovascular diseases. Genetic analyses of the strains developed in Japan and New Zealand have revealed a polygenic mode of inheritance (81,98). The primary mechanism producing hypertension is unknown; nevertheless, the hypertensive rat strains provide useful tools for studies of basic mechanisms of hypertension and for pharmacologic studies on antihypertensive drugs.

Major factors predisposing people to the development of atherosclerosis include dietary factors, cigarette smoking, hypertension, and physical activity, all of which may be simulated in animals. Factors implicated in the development of atherosclerosis include endothelial damage, smooth-muscle-cell proliferation, and high serum-lipid levels (85). Recent reports indicate that interaction between environmental factors, such as stress and diet, and high frequency of familial hyperlipidemias, which appear to be monogenic, may be responsible for the apparently increasing incidence of coronary heart disease (34).

Several animal models have been developed for the study of atherosclerosis in people (13). Criteria for the ideal animal model include rapid, inexpensive, experimental induction of the lesion with minimal experimental manipulation; sufficient animal size to provide tissues for multiple studies; and development of both morphologic and biochemical lesions similar to the human ones (91). Currently, no animal model fulfills all these requirements, but some existing models do satisfy a large proportion of them (7,72). Experimental studies have employed rabbits, pigeons, rats, chickens, pigs, turkeys, and various subhuman primate species. All have been instrumental in advancing knowledge concerning human atherosclerosis and have been the topic of several reviews (14,19,85).

To better understand the pathogenesis of atherosclerosis, we must understand the metabolic alterations resulting in elevated lipids and hypertension, as well as the mechanism of endothelial damage and the factors necessary for endothelial repair. Experimental studies indicate that atherosclerosis is, under some circumstances, a reversible process (40,82,85). We need to know what factors arrest or reverse the change.

Slow Viruses

Certainly one of the most interesting and perplexing areas of current, degenerative-disease research is that involving the "slow viruses," so named because they do not "obey" some of the virus "rules." As defined by Sigurdsson (94), slow virus diseases are accompanied by prolonged latent periods, a chronic course of the disease, and host specificity. They seem to be "invisible" to the electron microscope, they do not appear to be antigenic, and they are resistant to inactivation by UV light, formalin, and heat. Furthermore, we have no rapid *in vitro* test for them, and we do not know their constituents. Some workers suggest that instead of nucleic acid, they may be replicating membrane fragments consisting of protein and/or polysaccharide. A recent suggestion by Diener (20) is that they may be viroids, which are known to cause certain plant diseases and which consist of self-replicating RNA molecules (63).

Four severe neurologic diseases, referred to as subacute spongiform encephalopathies, are caused by slow viruses: kuru and Creutzfeldt-Jakob in people, scrapie in sheep, and transmissible encephalopathy in mink. These degenerative disorders are of great interest because of their similarity and because all four have been transmitted to subhuman primates (54,105).

It is highly likely that some human cancers and certain chronic diseases, such as multiple sclerosis, are caused by slow viruses. All chronic and degenerative diseases, including aging, are suspected and under investigation. Other slow-virus diseases being actively studied include equine infectious anemia (43), Aleutian disease of mink (44), and lymphocytic choriomeningitis (LCM) (76). These diseases are both similar and dissimilar to those of people. Their major contribution may be to disclose basic mechanisms of prolonged viral persistence in the host.

Neurologic Disease

Chronic, human, neurologic diseases are of vast medical, social, and economic importance. Many have no recognized counterpart in the nonhuman animal kingdom. Comparative neurologists will be required to make great contributions in the future to help unravel causes and develop effective treatments for such human disorders as multiple sclerosis, epilepsy, senile and presenile dementia, schizophrenia, and other forms of abnormal behavior. While we maintain a diligent search for additional, valid animal models of human neurologic diseases, we must carefully examine the existing disorders and continue to be resourceful in developing new concepts and approaches utilizing existing animal models.

More intensive study of existing neurologic disorders in animals may reveal basic mechanisms applicable to human diseases, for example, the pathomechanism of old dog encephalitis and its relationship to subacute, sclerosing panencephalitis (59) and other congenitally acquired disorders of man. We need to delineate the mechanisms that produce demyelination, utilizing existing models such as canine distemper (107), infectious leukoencephalomyelitis of goats (15), and globoid leukodystrophy of dogs (29). Also, we must elucidate the mechanism of motoneuron destruction, which is observed in kuru and scrapie (39,56) and in the inherited, lower-motoneuron disorder of the "wobbler" mouse (8). Many aspects of common, clinical, neurologic disorders of animals, such as epilepsy in dogs, must be more thoroughly investigated. Furthermore, we must evaluate brain function more analytically in order to identify and assess animal behavior and neurologic diseases, especially those displaying minimum deviation.

Aging

Some may question categorizing aging as a chronic degenerative disease, but we who are aging do not. The most useful potential sources of aged animals are veterinary colleges and certain animal research facilities. The animals involved are chiefly companion animals—dogs, cats, and horses—that are usually kept for their full life spans rather than merely to the end of their reproductive lives or period of "usefulness." Another source of aged animals is artificial insemination laboratories, where valuable bulls are kept to advanced age. Some primate centers now retain animals to advanced age, and zoos can provide a few animals of assorted species for possible gerontologic studies.

These sources of animals, if properly utilized, could supply some necessary basic knowledge regarding aging. At present, about the only thing gerontologists can agree on is the paucity of data on aging; certainly there is little agreement on how species grow old. Abundant theories explain aging (25–27,93,102), ranging from the belief that cells have their own clock, which regulates the mechanism of aging (41,97), to the proposition that aging is regulated via the hypothalamic–hypophyseal axis and is basically a neurohormonal transmission problem (28). A great deal of work needs to be done in the latter area; since techniques are available for testing the hypothesis, it is a reasonable, if not a high-priority, goal.

We must more closely examine and identify other degenerative diseases of animals in order to establish the pathomechanisms involved. These disorders include the arthritides; degenerative, intervertebral, disc disorders; gout; and the collagen diseases, including disseminated lupus erythematosus, scleroderma, and rheumatic heart disease.

CANCER

Presently one of the most awesome and costly problems in our society is cancer (12). And this unfortunate state of affairs does not exist because of inattention. Cancer has been the object of considerable attention dating back almost a hundred years to when a Russian veterinarian named Novinsky transplanted a venereal sarcoma from one dog to another. Extensive work in the intervening period has shown viruses to be vertically transmitted from dam to offspring and has implicated them in a large number of animals, including subhuman primates (1,6). However, the belief is still widely held that people are different; many researchers are still skeptical that viral etiology is involved in any human neoplasia. Recounting some of the history may be instructive.

Relative to viral induction of tumors, the first comprehensive animal-tumor virus was described in 1908 (23) and was obtained from leukemia in chickens. Little serious attention was accorded the finding, however, because bioscientists did not consider leukemia a neoplastic disease. Peyton Rous (86) was the first to discover a virus in a solid tumor—a sarcoma in chickens. But this finding was thought to have little significance for people or other animals. Fortunately for Peyton Rous, he began his scientific discoveries early in life and managed to live a long time, so he realized a measure of recognition for this early discovery of a tumor virus by being awarded the Nobel Prize in 1966, 55 years after his noteworthy discovery. About two decades later, Shope discovered the first tumor virus in mammals in a rabbit papilloma (92); this didn't cause much of a stir either. Shortly after Shope's notable discovery, Bittner (9) reported a very significant finding relative to a filterable, extrachromosomal factor that induced mammary tumors in strains of mice with a high incidence of this disease (84). We now know a great deal about this agent; it is an RNA virus and appears to be a useful model. Further interest was generated 15 years after Bittner's observation when Ludwig Gross (38) discovered what Rous refers to as a "do-all" tumor virus in murine leukemia (87). This interest was understandable because leukemia, a disease in mice similar in many respects to the same disease in people, was virus-induced. But the discovery did not immediately stimulate carefully conducted experiments to confirm the work. In the decade that followed, however, similar murine leukemia viruses were discovered, and researchers realized that these murine viruses resembled tumor viruses in chickens. Even though this work was both interesting and important, it seemed that the majority of bioscientists regarded the virus-induced tumors in chickens and mice as genetically manipulated special cases with little relevance to the human situation.

Even with the very significant discoveries of the virus-induced, leukemia–sarcoma complex of malignancies in cats, a highly outbred species (46,51,95), questions were raised about its relevance to virus-induced leukemia in people. In each species—avian, murine, feline—a C-type virus with RNA as its genetic material was implicated in causing a leukemia–sarcoma complex. It was of some significance and considerable interest that the feline sarcoma virus also induced tumors in young dogs and primates. More recently, a C-type virus has been implicated in subhuman primate tumors (49,50,52,53,100,103).

Virus-induced primate tumors in apes are currently of great interest. At least 15 gibbon leukemias have been reported, and there may be many unreported cases since leukemic gibbons may not show overt signs prior to death. Thus, the disease may go unrecognized unless the animal is thoroughly examined (T. G. Kawakami, personal communication, 1975). Studies of the reported cases indicate that the virus infection was not obtained from a single or common source (Kawakami). The largest number of leukemia cases was reported from one colony of about 200 gibbons that had been subjected to experimental conditions, but several cases were described in gibbons not exposed to experimental conditions (21,74, Kawakami). This suggests that gibbons do have spontaneous leukemia and appear to be an interesting model. It is of some significance that a virus recently reported in a human lymphosarcoma is closely related to the primate tumor viruses described above (31,32). Virus-induced tumors have also been reported in animals and people, implicating DNA viruses. But they will not be discussed here since the RNA virus serves as a reasonable paradigm for discussion purposes (24,64).

CHOOSING THE SPONTANEOUS MODEL

In selecting spontaneous models, we must evaluate their overall fidelity as well as their distinctiveness and reproducibility, since even the most carefully designed experiment can be vitiated by an animal model lacking in integrity. By fidelity, we mean their overall faithfulness to the human condition; by distinctiveness, we mean they possess a distinguishing characteristic that mimics a particular property in the human disease entity; and by reproducibility, we mean that sufficient numbers of animals can be produced to adequately study the disease. We often assume that primates are phylogenetically closer to people than other mammals are and that, therefore, they must be the best animal if they present a spontaneous model. This may or may not be true, depending on what is being studied. Species have been utilized with little or no discrimination: Sometimes an investigator has used the species before and is familiar with it; or, it is too much trouble to learn about alternate but superior animals; or, one species bites and another does not. Frequently, experimental animals are altered by exogenous materials or by surgical intervention, not because these are the best ways to approach the problem, but because they are the easiest ways.

It is doubtful whether any single species can provide all the answers necessary for extrapolating effects directly to people. Any animal experiment must be viewed with several questions in mind if the investigator is to make an enlightened choice of species. The investigator must decide what range of biologic responses (endpoints) will be considered important in the matrix of the experimental design, and he must carefully weigh the numerous and important cost factors involved. In the long run, small animals do not cost less just because they take up less space. Some of the critical cost factors are intangible, such as credibility of the data and the number of "bits" of data obtainable from each animal. The animal's life span is an important factor, particularly in studying cumulative effects of low doses of an agent. The experiment is abortive if the animal dies before the effect is apparent. The best test systems occur naturally and thus most closely resemble or mimic the disease to be evaluated. We do not demean the contributions made to biomedical science by using exogenous factors to produce a disease state, but, as we look to the future and grow more knowledgeable about the mechanisms of disease, we will benefit by having more than approximations.

The disease state to be evaluated should be well understood by the investigator, and this knowledge should be recalled in choosing the experimental animal that can best answer the questions posed in the experiment. The more "real" a situation is, the more likely it is that the results produced will be transferable across species lines for the benefit of both people and other animals. This is probably most apparent in the case of genetic diseases, where multiple instances of diseases identical in people and animals have been studied. In most, the enzyme defect and metabolic pathway are identical, and one can readily see the usefulness of this type of information from the standpoint of both diagnosis and treatment. Surely information obtained from animals in this manner is more valid than information obtained from other animals in a more contrived manner, whether they are farther away from or closer to people on the phylogenetic scale. Relative to reproducibility, sufficient numbers of animals must be available so the "reality" can be recreated enough times to allow analysis of the problem. This point is attacked in challenging the validity of the use of naturally occurring diseases. We can only respond that, in most instances, the animals can be nurtured and the diseases reproduced; when this happens, the results are gratifying.

PHILOSOPHY OF ANIMAL RESEARCH

The nature and extent of direct human experimentation will be reduced in the future for several reasons. Linda B. Behrens's (5) revelations of experiments on what she calls "kamikaze human guinea pigs" has stimulated legislation to protect human subjects. The elimination of the military draft, with the subsequent lack of conscientious objectors as volunteers, and the public outcry against the use of prisoners and institutionalized patients (69,73) have reduced the extent of human experimentation. This would suggest a great increase in animal experimentation and a need for more and better spontaneous models.

It is especially incumbent upon all of us who use animals in research to realize fully the heavy moral responsibility that we bear

(11,55,62,70). Before we do an experiment, we must ask: Is this necessary? What kind of pain and anxiety will the animal suffer, and can it be reduced or eliminated? Must the animal remain conscious for the experiment? Could the number of animals be reduced? Could tissue culture and computer models substitute for at least some of the animals (30)? In this regard we are in sympathy with the three R's of Russell and Burch (89). These three R's are:

1. Replacement by substituting insentient material for conscious, living higher animals.
2. Reduction in number of animals to obtain the desired information with adequate precision.
3. Refinement by decreasing the incidence or severity of manipulative procedures that cause discomfort to the animals utilized.

We must always be aware of the consequences of our acts. We must ask ourselves if we are fulfilling our obligations to the animals in our care. No unnecessary pain or stress should ever be inflicted. Furthermore, we must ask if our manipulations will result in important medical knowledge or information that will result in alleviating suffering and in prolonging or saving useful life.

We in comparative medicine realize and emphasize that the principles of disease should be studied more readily in animals than in people. In general, animals are more revealing since they can be specially selected for certain traits and/or are often inbred, which itself leads to interesting observations. Another advantage of animals is that they are confined to limited areas in concentrated numbers and are fed and handled differently than in their undomesticated state. This controlled environment enhances the chances of disease expression and makes it possible to recognize the disease state and follow its development. Early observations of the physiopathology of the disease process, which often are the most important, are possible in animals but precluded in people.

CONCLUSION

Progress in medicine has been noteworthy in our generation because we were successful in understanding the causes of a large number of infectious diseases that have a short incubation, acute onset, and relatively short duration and that are characterized by focal pathology. This knowledge led to the control of many of them, using the broadly accepted and effective techniques of antibiotic therapy and immunization.

As a direct result of curing the relatively "easy" disorders, we have revealed the diseases that are subtler in their onset, slower in their development, more devastating in their expression, and more protracted in their course. We are in the process of determining the origins of these disorders and are finding, to our dismay, that treatment of these conditions does not yield the results we have come to expect from our efforts in the forties and fifties. We have literally solved the problem of poliomyelitis in our generation, but atherosclerosis, coronary heart disease, slow viral infections, degenerative neurologic disorders, cancer, and, most of all, aging have failed to yield the necessary knowledge for us to provide solutions. Unfortunately, we must look forward to protracted, in-depth studies of these disorders before solutions are likely to be found. Studies of naturally occurring animal models will provide the mechanism for condensing the study period and elucidating these tragic disorders.

We must, of course, admit at this point that we are only beginning in our utilization of animal models. The concepts of "One Medicine," "Nature is a Unity," and the use of animal models for comparative medical research have been philosophically proposed by a long list of eminent scientists. However, the use of *bona fide*, naturally occurring animal models of spontaneous, human disease is still in the infant stage, demanding careful haste and continuing encouragement for beneficial maturation. We are just tuning the instruments prior to beginning the overture of a symphony that will have many movements. It gives promise of benefiting many creatures, great and small.

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DISCUSSION

ORLANS: I would like to have suggestions, please, as to what practical steps you would suggest to all of us as to how we can improve the dialogue in future years. I would ask Mrs. Stevens to speak on behalf of humane societies and Dr. Bustad on behalf of the biomedical community.

STEVENS: I will try to avoid a controversy, and then you can carry on from there. The two thoughts that have come to me quickly, and I must say I wasn't prepared for this question, are first: that all journals in which animal experiments are published ought to require a very clear description of exactly what was done to the animal, exactly what the dosage of anesthetic was, and if there was any variation in the type of anesthetic, that should be included. Postoperative care should be described, including drugs used for relief of pain. It should be completely clear what was done to the animal, quite apart from what is normally included in scientific papers. I think that would be a very simple and absolutely cost-free way in which real cooperation could be carried forward.

Second, I would like to suggest a very expensive way of cooperating. That would be to ask of our government a very large appropriation—rather an authorization first and an appropriation second—for work on further development of the use of alternatives to laboratory animals in a whole variety of ways. If more tissue culture work were being done, for example, it would attract more high-powered brains to develop more alternatives.

I won't give a long list, because we are going to hear papers on many different types of alternatives. Those seem to me to be two practical steps that could result from this symposium that certainly should be non-controversial.

THURSTON: I would like to back up Mrs. Stevens in that we need funds in every country for the development and promulgation of alternate methods to live animals. We also should call on the United Nations for a means of exchange of information on alternatives.

BUSTAD: I feel that the inclusion in publications of the significant data on animal care is very important. Perhaps the many editors of journals who are here today can help do something about this. My experience here has certainly been educational, and I will learn more as we progress.

I think a dialogue must be maintained. I am not here to suggest, at the drop of a hat, the format that this dialogue should take, but I think it is very important to have it, to try to reach an understanding, at least on our common objectives. You have hit on a very important one: to obtain more financial support. We should look at alternatives, look at refining the three R's that I mentioned in my paper, look at methods for improving what we have and developing better animal models and better housing. These, we recognize, are expensive. Hopefully, together we can set certain priorities. Our dialogue is exceedingly important in this regard and so is maintaining a respect for one another's position.

CORNELIUS: Just one comment concerning using alternatives to whole animal experimentation. I think this is a good idea. One of the problems you have in doing research on systems of disease is that, many times to get to the heart of a disease where you have some impact, one must really work on cell systems, sometimes isolated cells. You must work on perfused organs. Sometimes it takes a variety of different systems, and to think about stratifying and only allowing research on one type of system would somewhat stranglehold the open approach of solving a major disease.

I think that one should certainly be aware of the fact that we should attempt to use animals in a very careful way, but it is very difficult to tackle a disease problem and be limited by the types of methods you can use.

I think that idea of having special funding for just tissue culture or putting more money into one area to stimulate that type of research may be one way to get something done. But it could really stranglehold the scientific community on solving a problem.

THOMSEN: The speakers this afternoon have said several times that animal models had to be used because of the nature of the experiments. Presumably, people could not be used because they would be injured or hurt in some way.

I am not an antivivisectionist, but I get a lot of letters from them and humanitarians who say they are not antivivisectionists but who question the morality of that position. They would say it has been stressed that animals and people have similar instincts. One paper was excellent on that. It is pretty hard to differentiate except in terms of degree. The question is why is it alright to use animals for experiments that admittedly would be better done with human beings, merely because the humans would undergo some suffering?

We use "winos" and others to give blood at blood banks. I have no doubt it would be possible, by spending a little bit more money, to obtain volunteers and pay them something to undergo experiments. I would like to know your answer. I do not take this position, but I would like to know the scientist's answer to that question, which I get repeatedly in letters. People do not understand the philosophy that it is all right to use animals for these stressful or painful experiments, but it would not be to use humans.

CORNELIUS: I will try to answer that. Why should a human be subjected to or not be subjected to things that animals are? That is the question he is bringing up.

I think it is a matter of whether or not you are a heavy believer in reincarnation, and maybe the animal was you "coming back." Personally, I feel that there is a little bit of superiority of the human race over the animal race. I think if animals are handled in a humane way, they can help mankind as they always have throughout the centuries as companions. I do not think it necessarily means that we are going to take these poor animals and put them through inhumane treatment. I think man is at a higher level, from a central-nervous standpoint, and deserves better treatment than an animal.

BUSTAD: We can't assume, of course, that all experiments are painful. For example, in my discussion I said that we have observed hemophilia in many colonies of animals. We can do prospective studies on these. We can't very well do prospective studies on humans. Usually the disease manifests itself, and then we have to do retrospective studies, which are very difficult. We cannot, for example, determine if it is an autosomal recessive, that it is inherited, or discover the mode of inheritance. We can't very well pair up people and say, "Please reproduce." But we can mate dogs to find out the mode of inheritance and then say, "Well, this trait is inherited in this certain way. Therefore, certain pairs must not mate." Then we can extend genetic counseling to people.

I have been the subject of several experiments, and I felt a certain amount of discomfort was necessary to arrive at certain results. I operate under the premise that if one can obtain necessary information, even at some discomfort to me or my animal, that results in the saving of many animals, the discomfort is worth it. The well-being of a few is sacrificed for the well-being of many.

CAROL M. NEWTON

Biostatistical and Biomathematical Methods in Efficient Animal Experimentation

INTRODUCTION

Efficient experimentation is the aim of all able researchers. To learn the most from the least number of experimental subjects saves money and effort, and in some cases it also may save time. These and other considerations motivate, as well, the use of biological preparations below the level of intact animals, whenever the questions being addressed can be answered by doing so. Cellular cultures and biochemical preparations tend to be less complicated by inherent biological variation and amenable to more controlled experimentation than intact animals. Measures on them may be more quantifiable, and they may at times relate more immediately to the mechanisms being studied. Thus, other reasons join humane considerations to motivate efficiencies in animal experimentation, both by resorting to it only when necessary and by designing experiments as effectively as possible.

The theoretician's role is to help derive the most information and insights possible from experiments that have been done, sharpen strategies for future experiments, and direct the results of such work as rapidly and effectively as possible into dependent areas such as medicine. Clearly, these goals are shared by the experimenter. Ideally, an effective interplay between theory and experiment should inhere in each researcher. At present, there is a division of labor whose productivity depends somewhat on the understanding each has of the other's work.

BIOSTATISTICIANS AND BIOMATHEMATICIANS

Statisticians develop and apply methods for analyzing the results of experiments or surveys that provide an assessment of what information may be derived from them, of the certainty with which certain conclusions may be drawn—e.g., the likelihood that they could not have come about by chance. They also study how experiments should be designed to reduce the effect of certain uncontrollable sources of variation, to permit effective techniques to be used in their analysis, and in general to obtain the most information with greatest certainty in the shortest time using the fewest subjects. The ability to realize these goals, and hence optimal approaches to doing so, depends somewhat on one's area of experimentation.

Biological variability, missing data, and the problem of unknown interactions underlying measured variables are among the challenges addressed by the biostatistician in his own research and in his collaboration with laboratory or clinical investigators. In general, his attention in each design or analysis tends to be directed to one experiment or a very closely related sequence of similar experiments. He also inclines to develop methods requiring the fewest, most likely realizable assumptions about the underlying biological mechanisms. Thus, although the details of all underlying cellular and molecular mechanisms by which two drugs act in treating a given type and stage of cancer may not be known, the biostatistician can provide reliable, efficient experimental designs and analyses for determining if one drug results in significantly longer survival times than the other.

The biomathematician's focus complements this. He is concerned about designing and analyzing experiments to better understand what the basic biological mechanisms are. He supports this growth of understanding with mathematical or computer models that portray his conceptions of the systems being studied. In general, these models seek to bring together what has been learned from a number of quite different experiments. As he fits onto the framework of his models the pieces of information derived from each of these experiments, he sometimes finds that there is an essential conflict between one model and the pieces of information. If the latter survive a careful second scrutiny, perhaps involving confirmatory experiments, the model must be rejected.

During the investigation of a certain biological topic, more than one model, i.e., more than one conception of the underlying biological mechanisms, usually is being entertained. Ideally, one selects experiments that are likely to definitively narrow down the number of possible alternatives. Very often the experimenter can do this without theoretical supports for his conceptualizations. However, in the complex situations often encountered in investigating biological systems, especially those in intact animals, those supports can be very helpful. Comparisons of how existing facts fit several alternative models help to point up where missing information appears to be most critical. Furthermore, the effects of several experimental approaches can be compared by exercising the models being entertained—to identify those experiments whose results are likely to be most strikingly different for different models. Insofar as our knowledge is incomplete, these assessments must be more tentative. But even in early phases of an investigation they are likely to improve upon random probing, and this likelihood grows as one's understanding of the system grows.

One certainly must remain mindful of the risk that the "correct" model is not among those being considered, attempting at all times to discover new possibilities consistent with known facts, which should be added to the group of contenders. This perception of a possible new theory has long been recognized as one of the most creative aspects of scientific discovery. Formal mathematical- or computer-modeling efforts can only help prepare the way for it and facilitate its translation into scientific endeavors; they cannot replace it.

In summary, the biomathematician's mathematical or computer models attempt to portray currently held concepts about how and why a biological system functions. As facts from a number of very different experiments are related to these models, some models will be rejected, and the remainder may be exercised to suggest what future experimental probes might most effectively distinguish between them. Several aspects of this endeavor might reduce some needs for animal experimentation: A model may effectively bring together information from many different levels of experimentation. One can try out ideas for utilizing a combination of experiments below the intact-animal level to distinguish between competing theories. And, in those cases where the nature of questions to be answered must of necessity require animal experimentation, sharper strategies for pursuing such experimentation would seem likely to reduce the number of experiments required, and hence probably the number of animals required, to attain a given level of understanding.

Although modeling to better understand biology is the activity most characteristically associated with biomathematicians, they make other contributions to biomedical research. For instance, they may develop the mathematical or computer components of new experimental techniques. Also, as will be illustrated later, they may develop models for exploring new strategies for treating disease, based on knowledge derived from a variety of biological experiments.

Closer communication and greater overlap of the work of biostatisticians, biomathematicians, and biomedical researchers should be encouraged. The biostatistician often can provide a better design for a given experiment if he better understands how it fits into the total research strategy. As biomathematicians or experimenters seek to sharpen these strategies, it helps to know what experimental designs are possible and most efficient.

EXAMPLES OF STATISTICAL RESEARCH

Screening for Carcinogens

There is concern that mild, continuous exposure to certain agents in our environment and diets may contribute to our susceptibility to cancer. Because the mechanisms for this are not fully understood, it is not possible to identify many potentially hazardous agents without recourse to experimentation. Various approaches seek to narrow down to the agents of greatest concern prior to experimentation on animals. Even so, large numbers of these now require such testing. It is not feasible to test all of these at once. Each experiment requires many animals, because the expected yield of cancer is likely to be low, and it requires a long time for that yield to be expressed. This research is expensive, and, for the public welfare, it is urgent. Biostatistical research on strategies for sharply reducing the number of animals needed and accelerating the overall flow of information about carcinogens has been supported by the National Cancer Institute. It has led to some very promising approaches, such as a recently reported two-pass procedure. One of the biostatistical investigators for the latter, Dr. Robert Elashoff, is preparing a review article on screening procedures that should be available soon (2).

Savings from sophisticated designs can be substantial. Consider the following comparison of a traditional one-step experiment for assessing a chemical compound's capacity to induce cancer with the new two-step procedure mentioned above: In the one-step procedure, a large number of animals (N_1) are divided into treated and control groups. After a specified period of time, the numbers in both groups that have evidence of tumor are assessed, and this results in a score that decides whether or not the compound is carcinogenic. In the two-step procedure, a smaller number of animals (N_2) are divided into treatment and control groups, assessed for the presence of tumor after the same period of time, with a resulting score that places the compound in one of three groups: strongly carcinogenic, weakly or questionably carcinogenic, or not carcinogenic. Further testing by a one-step procedure is required only if the score is in the middle range. Because the first step of this two-step procedure is not required to yield a definitive yes–no answer, N_2 can be substantially smaller than N_1. Dr. Elashoff indicates that, for final assessments of equivalent certainty, a one-step test requiring 600 animals might very well be comparable to a two-step test beginning with 180 animals. Numbers of animals assigned to treatment and control in the two-step experiment can be chosen to regulate the likelihood that outcomes will be in the middle range. This can be held down to as low as 15 percent. For the example cited, Dr. Elashoff estimates that the total average costs of both types of experiment would be about $150,000 (one-step) and from $100,000 to $125,000 (two-step). These costs are very closely associated with the numbers of animals required. In addition, a large number of potential carcinogens are awaiting testing. From the figures indicated above, two or three compounds could be tested simultaneously by the two-step procedure, with some savings on controls, in animal facilities adequate for the testing of one compound by the one-step procedure. Although the final answer would be delayed for questionably carcinogenic compounds, the overall flow of information would be greater for the two-step procedure. Essentially, the two-step procedure economizes by focusing resources on the most difficult assessments, those of questionable carcinogenicity. The concept is straightforward, but sophisticated biostatistical research has been required and is continuing to further explore and guide implementation of this type of approach. The National Cancer Institute should be commended for encouraging this kind of research, whose costs are modest compared to savings in animals and dollars that can be realized in subsequent carcinogen screening.

Sequential Designs

For many years statisticians have been researching on experiments that are designed so that some decisions concerning their conduct are made during the course of the experiment, contingent on what information is available at the time (5). For instance, a stopping rule may be developed for terminating the experiment as soon as a conclusion can be stated to a given level of certainty. Such an approach tends to reduce the number of subjects required, but it may take longer than alternative approaches. For example, to determine the LD_{50}, the dose of a given agent that can be expected to produce a defined effect in 50 percent of the subjects being treated, a sequence of experiments can be run with as little as one subject each. The dose given each subject is determined on the basis of the outcome for the last, and a predetermined stopping rule is adhered to (1). This approach is very efficient with respect to the number of subjects committed, but it may be slow if considerable time is required for the defined outcome to become manifest.

One anticipates an increasing role for sequential designs in clinical research that compares the efficacy of various treatment alternatives. Patients are admitted continually to such a study, with random assignment to different treatment alternatives as in conventional designs or perhaps with some modification as the study progresses. Frequent analyses of outcomes to date are scheduled as part of the study design, and admissions to the study are terminated as soon as the conditions of a stopping rule are met. This approach substantially alleviates ethical concerns about assigning some patients to less-satisfactory treatment alternatives; the study continues only so long as a significant difference between alternatives has not been found.

Computers in Biostatistical Research

Biostatistical research is very active in the foregoing areas and in many other approaches to efficient experimental designs and powerful analytical techniques, in extracting the most information possible from well-designed studies. Much of this research today can reach beyond the limits of exact mathematical methods by employing computers. For instance, computers can be used to simulate many replications of a type of experiment under given assumptions about important factors such as the variance of certain components, causes for data to be missing, and so forth. Different proposed methods of statistical analysis can be compared and further refined with respect to their reliability and efficiency in extracting the appropriate information from these simulated experiments. To establish these conclusions on firm ground, the simulations usually must be rather extensive and probably would not be attempted without computers. Although this computer-aided statistical research may at times be expensive, in all likelihood its costs can be recovered many times over by subsequent savings in laboratory or clinical research.

Computers also are important aids to implementation of some of the new experimental designs, e.g., the frequent analyses required for sequential clinical trials. They also make it feasible to apply sophisticated statistical analyses to more conventional experiments. Before computers were available, some of the most effective of these analyses required a number of days to perform by hand for a typical set of experimental data. Needless to say, their practical use was limited, particularly for experiments with very large sets of data, where they probably were needed most. Computers and the statistical programs developed for them (4) have transformed this situation. All investigators now can have prompt access to sophisticated statistical tools at little cost and to analyses that can extract more information from their experiments. Relieved of time-consuming hand calculations, the consulting biostatistician has been able to serve far more investigators better and at less expense. He has had more opportunities to gain experience in the use of advanced statistical methods and often more time to contribute to their advancement. New experiments can be designed with knowledge of results of preceding experiments shortly after the latter have been completed. Thus, statistical supports have become infused far more widely into biomedical research, and this very likely has contributed to higher standards for acceptable scientific reporting, as well as a higher and more reliable yield of information from the experiments that have been performed.

EXAMPLES OF BIOMATHEMATICAL RESEARCH

Understanding Mechanisms of Respiratory Control

A number of centers in the nervous system communicate with the pulmonary and circulatory systems to regulate respiration. Although much is known about this highly complex and medically important function, much work remains for the basic biological researcher. Some of the experiments upon which our current knowledge is based have not required the use of intact animals; others of necessity have. To judge what best to do next, we must intelligently piece together all of this information from highly diverse experiments. This is a job for the biomathematician or biomathematically trained biological researcher; deep and comprehensive biological scholarship obviously is required. William Yamamoto is such a scholar, and his recent article on the interplay of biomathematical and laboratory research in the investigation of respiratory control (6) should be read in entirety.

The biomathematician's role in effective biomedical research is not well understood by many people, perhaps because not enough biomathematicians have been appropriately trained for this role, i.e., to be leading biomedical scholars with great strength in mathematics and computing. In essence, once it has been decided that a certain experiment should be performed, the biostatistician may help to reduce the number of subjects required to obtain a given answer. The biomathematician may substantially reduce the number of different experiments to be performed by identifying from among many possibilities those that strike most effectively toward the basic issues one seeks to understand.

Exploring Strategies for Cancer

Models can be used to study better ways to treat certain diseases. One example is a system that has been developed to aid in translating what has been learned from research on both cellular systems and intact animals into the exploration of effective strategies for treating cancer (3). Many plausible ideas have been tried out on this computer model during the past several years. In certain areas of the biological system for which definitive information is lacking, the model has been rerun many times to cover a wide range of possibilities. One treatment strategy, a carefully timed sequence of neutron and X-ray therapy, appears to be especially promising. We now can go no farther without animal experiments to confirm this prediction. They are necessary because the cellular control systems being exploited by this treatment strategy are not fully understood, and because cellular cultures are not yet capable of portraying these control systems. However, our experimental focus is far sharper than it would have been several years ago.

It is more difficult to quantitate the savings in animals, money, and investigators' laboratory efforts that are realizable from biomathematical research of this type than to do so for the biostatistical approach to screening carcinogens mentioned earlier. However one suspects that it can become considerable, especially if biomathematicians are well trained and identify as biologists and if theoretically strengthened biomedical curricula alert laboratory investigators to what may be realized from preliminary modeling. Some illustrations from the program for exploring cancer treatment strategies may serve to impart a feeling for how various fragments of research information can be usefully brought together into a model and how more definitive planning for subsequent laboratory research can be accomplished. They also are examples of interactive computer graphics, an especially powerful technique for exploring models.

Much of the information requested by the program on cell sensitivity at various parts of the cell life cycle may be derived from experiments on cellular cultures, as are, to a considerable extent, the general dose–response functions. On the other hand, experiments on intact animals were required for information on how the body's cellular systems respond to injury—i.e., on the controls that regulate a given tissue system's commitment of more of its cells to the path toward cell division if its population has been depleted by injury. Notice that the model is a framework into which this experimentally derived information may be fitted, for examination of its completeness, consistency, and consequences. The model itself imposes as few assumptions as possible. The most important assumption here is that malignant cells lack the aforementioned active controls for regulating population size.

A number of different treatment strategies were examined on this more elaborate modeling system. When one emerged as being especially promising, a simpler, specialized model was developed to explore it more intensively and economically. By this time, the most important factors bearing on outcome had been identified. This model also has been implemented in an interactive graphics mode, which permits its flexible exploration with immediate graphical feedbacks. Figure 1, a photograph from the computer terminal's display screen, compares responses of "normal" and "malignant" cellular systems to a sequence of paired irradiations by neutrons (*N*) and gamma rays (*G*),

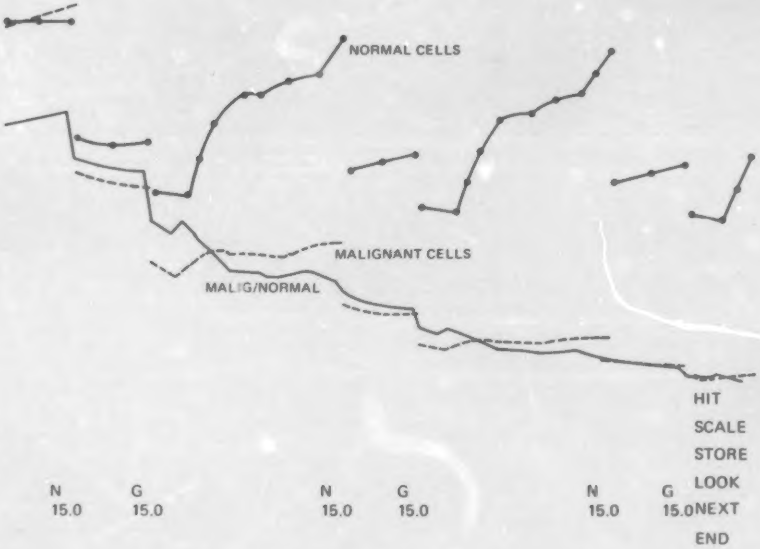

FIGURE 1 Strong feedback for normal tissue.

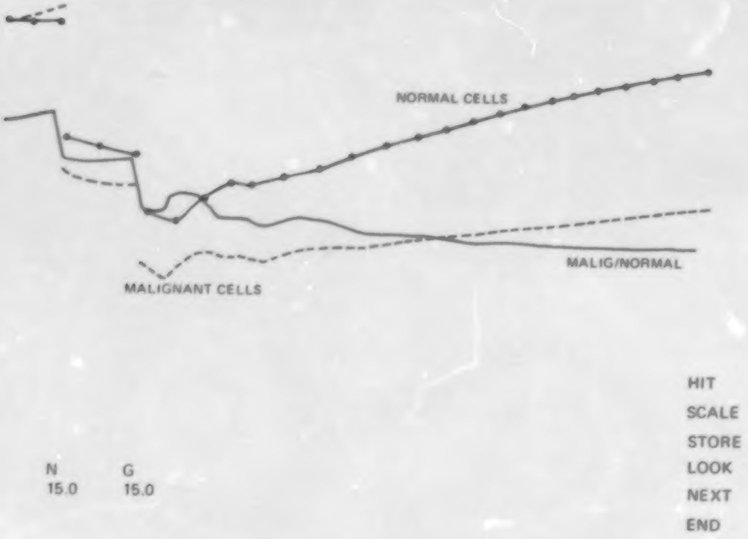

FIGURE 2 Weak feedback for normal tissue.

the latter following the former by about 8 hours. Subsequent treatment pairs are initiated after the "normal" cells have repaired. Figure 2 is the same, but the normal cellular system's population-preserving feedback is far less active.

Note the great importance of learning more about these control systems. Other illustrations show that timing between members of the treatment pair is important, as has been observed in split-dose laboratory research with gamma rays; cells recruited for subsequent division pass through a phase in this preparatory cycle in which they are relatively immune to gamma rays. Effects of neutrons are less dependent on the cell's location in cycle, and exploration of the model indicates a possible advantage of neutron–gamma sequences.

What does this all add up to? By modeling, we were able to make more effective use of individual items of information we already possessed. Insofar as we have enough knowledge, we can readily explore the consequences of various proposed treatment strategies based on it, narrowing down to what seems most promising for subsequent, definitive laboratory research. Yet, while this kind of preliminary study may serve to reduce the requirements for animal experimentation, it cannot stand as a substitute. We now must design careful laboratory studies of paired neutron and gamma effects on normal and malignant tissues in intact animals.

Computers in Biomathematical Research

Here, as in the case of biostatistics, computers have enabled us to reach beyond the limits of what is possible with exact mathematical methods alone. Portrayals of complex biological systems frequently are not amenable to useful mathematical descriptions without simplifications that sacrifice important aspects of their realism. Many of these systems can be described by flexible computer models that facilitate their modification and the investigation of their responses to a variety of experimental interventions.

Statisticians have provided methods, such as regression, for fitting a mathematical model to a set of data from one experiment. There are at present no standard methods for fitting together pieces of information from a number of quite disparate experiments, relating them to various concepts of how the underlying biological system functions. To discover such is a fundamental aim of biomathematical research. At present, modeling must be conceded to be as much an art as a science. Hence, heuristic aids to the investigating mind must be devised. Interactive graphics is an especially promising approach to this (3). It provides the researcher effective graphical portrayals of the model's status while computations are in progress, permitting him to freely manipulate the model and immediately observe the results of doing so. It also facilitates collaborative modeling by biomathematicians and their colleagues in laboratory research.

PROBLEMS

The possible contributions of biostatistical and biomathematical methods to biomedical research are far from being fully realized. Among the problems contributing to this (4), two deserve special mention.

An ultimate barrier to use of any aid is lack of awareness of what it can contribute and how it can be procured. While there appears to be a growing tendency to include some minimal biostatistical training in medical curricula, not many biological or basic medical sciences require this or any significant biomathematical training of their doctoral students. One very likely reason is that few appropriate courses have been developed for them, courses that include an interrelated blend of computer, statistical, and modeling methods with primary emphasis on their application to real biomedical problems. Biology's evolution into a quantitative science with important theoretical components is now well advanced and progressing rapidly. I believe that there is a need for undergraduate biological education to more adequately reflect this, as has long been the tradition in undergraduate education in physics.

In earlier discussions it was mentioned that, to be truly effective in their work, biomathematicians should be generally well trained in biology, deep scholars in their biological specialty, and expert in the mathematical and computer methods that enable realistic modeling of biological systems. The myth that a mathematician cannot become a first-rate biologist, or that a biologist cannot become highly competent in the application of mathematics and computers to his research, has been disproven. Many gifted people have talents in more than one dimension. Yet, biomathematicians of the type described above are in very short supply, especially if biomathematical upgrading of undergraduate biological curricula is to be attempted. We should actively seek to identify and appropriately train top students for this field. We should provide them adequate support for the long and very demanding graduate training required to equip them for truly productive, effective biomathematical careers. There can be no compromise in the excellence of both their biological and mathematical preparation, as well as additional training in statistics and computer methods. Many additional hours of individually guided instruction and research are required to mature them in the art of modeling and in other aspects of synthesizing their knowledge of biology and effectively deploying their armamentarium of theoretical tools to help advance research in their biomedical specialty.

CONCLUSION

Properly trained biostatisticians and biomathematicians can contribute substantially to improving the efficiency of animal experimentation, but they cannot fully replace it by theoretical devices. Computers are important to the advancement of their methodological research, as well as to the application of its results to research in biology and medicine.

As part of their graduate training, biomedical researchers should be made aware of how computers, biostatistical supports, and biomathematics can aid their research. Then we must ensure that these aids will be at hand. Adequate computers and terminals, reliable and well-maintained statistical programs, sophisticated modeling systems, and appropriately trained biostatistical and biomathematical consultants or collaborators are important elements in this support.

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DISCUSSION

COPE: I am a physician, animal experimentalist, mathematical biologist of 15 years of experience, and Director of the Society of Mathematical Biology. I would like to say that my experience confirms and supports what Dr. Newton has been telling you—that mathematics and computers can be a great aid in the analysis of experimental data. I would like to state again some things that she has already told you, with some increased emphasis. The use of computers and mathematics in biological research involves the analysis of data that come from animal experiments. It leads to predictions that may or may not be right and therefore have to be tested in animal experiments.

It may also provide greater efficiencies and speed in the analysis of these data, which will save great time and may save the necessity of some other animal experiments. It may, however, increase your need for animal experiments because it gives you greater efficiency and speed. It shortens up the time between the first set of experiments, in which you get your original data, and your final predictions, which lead to the next set. With skillful use of computers and mathematics, you may end up using more animals and doing more experiments, rather than less, under some conditions.

While this may sound hard on the animals, it results in a speeding up of the research process, so you come sooner to your final result, which is to understand better how life systems work and perhaps to discover better treatments for diseases.

I want to emphasize that the computers and mathematics are an aid to animal experimentation; they are not a substitute. I think that this is the most important point that should be made.

LORD: I think that one question has remained unanswered. It concerned the use of human beings as experimental animals. I think we have completely overlooked the fact that there is legislation or regulation concerning this fact. With the passage of the Kefauver amendments in 1963, an item emerged that was known as "patient consent." Before any untested, new, or unapproved drug is used in human beings experimentally for the condition for which it is intended, that patient must give consent. This has not been too difficult with adults, but it has been a terrible problem with pediatric research. It is not the easiest thing to get a mother or a father to consent to using an experimental drug in infants.

The second thing is that I think society's concern for the welfare of the individual has led Senator Ted Kennedy to introduce some new legislation concerning the use of human beings in experimentation.

GAY: New legislation was just introduced in Congress to extend the life of the National Commission for the Protection of Human Subjects, which is currently meeting. Earlier they placed a moratorium on fetal research, and this type of research has slowed down even though the ban was recently lifted. Scientists now find many more restraints on investigations involving children. The question that was asked earlier, about why use animals first, has become as much a question of public policy as a question with a strictly scientific answer. While there are several scientific reasons for using animals, they have not been brought out at this session.

SPIRA: Do animals suffer? I think that if we assume that suffering and pain are intrinsically bad, unless there is a real overwhelming, overriding justification for inflicting it, then it is bad for any sentient creature, whether it is man or animal. You can kick a rock, but if you do it to a dog, it will suffer.

I do not think the question is theoretical, whether animals do or do not suffer. If they have a nervous system similar to ours and they have a similar origin from a common evolution, reactions will be the same. If somebody puts a lighted cigarette on somebody else's face, I do not feel it. But I assume that there is pain, because I would feel that pain. If I were confined, I would feel the pain; I assume animals feel similarly.

I think that if the experimenter feels that he can do this experiment in good conscience on a mentally defective orphan, then he can justify experimenting on animals. I think that is sort of the criterion. Otherwise, you get into a whole subjective, intuitive thing, where you intuit that humans are better than animals.

RACE: I would like to make two comments. First, it seems to me the computer creates a great instrumentation for efficiency, just in the handling of data. For example, the pathologist who counted the type II pneumocytes in 15,000 alveoli took 3 to 4 days to do it. With the computer it can be done in about 15 minutes on the image analyzer and printed right out. Data can be handled much more efficiently.

The second thing is that the efficiency of handling animal data is such that the example that I quoted in my paper on measurement and prediction of the osmotic gradients in the limb of Henle's loop was simply a computer adaptation to hard physiological, numerical data. The data had been developed in the past and was now being reexamined by the computer, giving one, two, or maybe three alternative hypotheses based on hard data already available. No further experimentation was needed, just permutation of the data properly. The computer came up with a couple of additional hypotheses that were not originally considered by the investigators.

Finally, I would like to comment on one thing that has wobbled through the discussion several times. That is, the use of dogs and various pet-type animals versus animals bred specifically for research.

Dr. Leo Bustad has published a beautiful article on the miniature pig. The miniature pig is not normally considered to be a household pet and certainly could be bred in large numbers for animal experimentation of a type that is highly applicable to higher mammal orders and to human experimentation. That, again, is an alternative for those experiments where we must use animals.

NEWTON: I agree completely with both of the people who spoke on biomathematics, and I thank them for bringing up these additional examples.

THOMSEN: What proportion of the schools of medicine and veterinary medicine would you say have departments similar to the one that you have, Dr. Newton?

NEWTON: Very few. There are a number of good biostatistics departments or biostatistics groups. There are biomathematicians working in biophysics and in some of the biostatistics departments, but there are very few departments that are primarily concerned with biomathematics. The oldest was the Committee on Mathematical Biology formed by Mirshevsky and Landall at the University of Chicago in 1935. There is considerable activity at the University of Alabama, in Birmingham; at the State University of New York, Buffalo; at the University of Chicago; and an excellent program at Chapel Hill, North Carolina. We are just beginning a new program.

You can see how easily they can be counted. In many other areas, such as the Latter-Day Saints' Hospital, there is considerable biomathematical activity but no degree-granting program.

THOMSEN: My second question is in what proportion of the in-house committees, the ones that review the proposed research projects, in what proportion would you say a mathematical statistician of some sort, a biomathematical statistician, or mathematician is a member of the committee?

NEWTON: I would like to let NIH answer this, because my sampling is biased.

THOMSEN: They do have departments of statistics, even though they may not be in the medical school that they could call on.

NEWTON: In all honesty I am only familiar with NIH procedures. So far, I have found them very conscientious with respect to biomathematical and biostatistical consultation in research. In fact, I think that the peer review system has militated this of necessity, because these are the kinds of questions that one would have to be asked. But I honestly do not know about other organizations. I think you have to get that information directly from NIH itself. I, myself, have been very impressed by their peer review system.

THOMSEN: My third and last question. You undoubtedly read the research journals of various kinds. What proportion of the projects reported on in those journals, and they do not include all the projects undertaken because in many cases they are unable to get a report published, would you say have been adequately designed from the standpoint of the biomedical statistician or mathematician?

NEWTON: Since I cannot give you absolute figures, I will have to give you relative figures. There is tremendous variability among the journals. I will not mention the name of one, but whenever we want to get a bad example for our medical students, because our medical students at UCLA are required to take some biostatistical training, I know right where to go. There may be other places too.

On the other hand, I have been impressed by the increase in quality of articles I have seen in a number of what I consider very solid research journals. I think there are a number of reasons for this. First, the biologist is becoming much more aware that this will help him get the most information. Second, thanks to some of our training programs, we have begun to train, not enough but at least more, people to go out and work with him. Third, because these people can get their hands on programs that are already written, they do not have to write them themselves to do sophisticated analyses.

I am impressed in a relative sense of an increase in the quality of articles insofar as I sample them, but I cannot honestly report in percentages.

THOMSEN: Would you not say that of the ones that you observed that half of them are adequate?

NEWTON: I am highly selective. If I quickly see that one has not got what I want or maybe has not gone on solid grounds, very often I will not proceed. I make that assessment far more often on the basis of the basic experimental design in the biological sense than I do in the statistical sense. Very often, statistics cannot repair a poor biological design.

THOMSEN: I think it is possible to see from these answers that the humanitarians, and some of them are looked on as a little "kookie" or radical in this field, who talk about reduction and replacement continuously show that they do have a real point. I think it can be demonstrated, with other questions and answers from various scientists, that there is no doubt at all that the quality of biomedical research is very, very poor compared to what it could and should be. These people, whom the scientists look down their noses at and say are radicals, have a very real point to make.

I think your paper, Dr. Newton, if you compare that with what you see in the journals, shows that very well.

NEWTON: I would like to respond that I think people of good faith are far in the majority in all aspects of this issue. I do feel that the biologists, not just for humane reasons, but actually for reasons of good science, are getting the most out of hard work in the laboratory. They increasingly are motivated to use good statistical and biomathematical techniques in research.

I think that the people in humane work are quite right to press for more responsible scientific work as far as animals are concerned, but again I believe this all converges. I think that the good quality of scientific work will meet the same objectives as the request for responsible work with animals.

TREAT: I think one of the most important points made by Dr. Newton's talk is that we are on the threshold of the twenty-first century. Most of the gentlemen had referred to the historical perspective in research. We are moving into a new century in a time when we are getting away from the methods that were used by physicians and researchers in the past. This is the time of a new modality. I am hopeful that computers will reduce the use of animals.

My questions to Dr. Newton are: Do you feel that by the time we reach the twenty-first century we will be getting very far away from the use of animals? Will we be breeding an animal with tissues that would be responsive to experimentation but that would in some way have a cortical sense that would not respond to pain? I know that at present certain nerves can be cut so that pain is not felt in human beings. This is done with people who have chronic back pain.

NEWTON: The second part of that question is outside the bounds of my competence. In the case of biomathematics, the answer is no, and let me tell you why. The biomathematician can only use what has been learned in the laboratory. He must utlimately go back to the facts that have been found in the laboratory as the roots, as the nourishment, for his work. Ultimately, he must test what he has found in his computer simulations back in the laboratory.

If the day does come when we can make a perfect computer model of any intact animal system, there will be no further point in studying that system, because we then will have had all the knowledge about it that we need. The computer and the modeling comes into play to help us to decide what we must do next to understand that system. But, if it is ever possible to make a perfect model, then we will have all the knowledge, and there will be no point in modeling.

HANLEY: I am as much for pigs as I am for dogs, actually. The possibility of using alternatives, promoting the use of animals other than those that people are emotionally attached to, may be consoling to some people. I do not think it is going to be a real alternative for those people in the humane movement, especially in the case of an animal like a pig, which is quite an intelligent animal. They are probably more intelligent than the dog, and thus they have probably a greater capacity for suffering.

We have a law in California that has worked out quite well that prohibits painful vertebrate animal experiments in elementary and high schools. It took about 5 years to get this bill through. When we would go for testimony, the greatest opposers of the proposal were the animal suppliers, who made it their business to maximize the numbers of animals that would be used in the schools or in research. They were very active in lobbying against any kind of reform, because they themselves had a financial loss.

In the case of computers and biomathematics, would you expect to find any resistance from the industry whose people make a business of using the maximum number of animals? Do you think they would offer resistance to a movement to find alternatives?

NEWTON: I think that many people, when they find their incomes threatened, do things that they might not do otherwise. But as to where they would take off, particularly on computers or something else, is a question I am just not qualified to answer.

COPE: I can speak directly to the point raised, hoping that in the twenty-first century methods would reduce the necessity for animal experimentation. Dr. Newton gave as a possible example the Navy development of a hormone that corrects stress for mitochondria. The work was done by my colleague, Dr. Polis, and I can tell you that it came completely from animal experimentation, mostly studying the mitochondria of rat liver, with further work *in vivo* in monkeys. Computers played a very small role, restricted to simple statistical analyses of the data. In no way does this example tend to suggest that animal experimentation will be less useful in the future for developing major medical advances. Quite the contrary, it suggests that animal experimentation will be at least as important and perhaps even more important. This work could not possibly have been done by any kind of substitute for animal experimentation.

FREE: Dr. Newton, we are here together to try to understand each other. I am wondering if the computer and all of your sophisticated systems can help close this gap a little bit more so we could understand each other more when we use the word "stress." We care just as much about the sentient pig or any other animals. It is not people out to rescue the animals that they identify with the most. I am wondering if you can do more to make us understand each other a little bit better?

NEWTON: I do not think the computer can do it as a mechanism by itself. It has enabled us to handle vast amounts of quantitative data faster and better. It has focused our attention on the possibility that we can in fact cope with this complex data base. I think the new generation of biologists, not because of the computer as much as because of their own instrumentation of various types that often is computer-supported, is embarking on a more quantitative orientation towards research.

Almost inevitably, this will imply that they will attempt to define their terms better. When one does define one's terms better and more exactly, one does communicate better. My feeling is that we all really have the same intent. I believe that good communication is going to be one of the things that will bring us together. Intent, though, is a human virtue, not a virtue of the computer.

WILLIAM GAY

Summation of Day One

I would like to close with three observations. First, is there anyone here who attended the 3-day meeting held in this very room in March to discuss experimentation on human subjects? If there is, perhaps you could talk with Dr. Thomsen and help him in answering his mail. That discussion had many points that were relevant to our discussion. All of the information from that meeting and from the Commission for Protection of Human Subjects emphasizes the need to do animal experimentation first before clinical trials. Perhaps Mrs. Stevens, who attended that March meeting at the Academy, could give Dr. Thomsen a little help on the problem of public policy here and perhaps help in answering his mail.

The second comment I would like to make is that there have been some alternatives to laboratory animals described for obtaining new biological information, and there have been many comments by the public on this subject. But I have heard few comments from the scientific members regarding the possible productivity of these alternatives. The scientists may want to add their information to the record tomorrow.

The third observation relates to questions by Dr. Thomsen and by Dr. Orlans about the future. Drs. Benirschke, Newton, Bustad, and Kramer made some comments about the future of the techniques they were talking about. "The Future of Animals, Cells, Models, and Systems" is the subject of this symposium, and I hope that we may direct some of the discussion tomorrow, and perhaps even some of the papers, to a little more of a look at the future. I do not think we have really discussed that and speculated about it as much as we should.

THOMAS S. FOLEY

A Legislator's View on Animal Legislation Affecting Biomedical Research

When I became Chairman of the House Agriculture Committee, I was nearly 46. Morris Udall once quipped that the House of Representatives was the only place where one could still lead a youth rebellion at the age of 46. This was on the occasion of his attempt to become Majority Leader of the House.

The Congress, of course, has undergone many changes recently, and among those now serving in the House are a total of 91 new members representing both political parties. Because some of the issues that have arisen previously have not yet been presented to the full House in this Congress, it is difficult to predict with any real accuracy what the general reaction might be. Further, the question of substituting *in vitro* techniques for experimentation on live vertebrates has never really been clearly presented to the Congress for its consideration.

In 1876 the British government enacted its Cruelty to Animals Act, which prohibited experimentation on living vertebrates without a license from the Secretary of State. Last year an amendment was proposed to that act requiring that no such license be granted if an alternative means of performing such research exists. That particular amendment was talked out, as they say in the House of Commons, in the report stage, but the attention it received indicates that the issue will probably be raised again.

To my knowledge, the only similar measure to come before the Congress was introduced recently by Representative G. W. Whitehurst of Virginia as House Concurrent Resolution 410 and provides: "Resolved by the House of Representatives, the Senate concurring, that it is the sense of Congress that the Federal Government should take appropriate steps to develop new research methods for its research projects where feasible, to complement or eliminate current methods involving the direct or indirect use of animals; and that no Federal funds should be provided for research projects involving the direct or indirect use of animals if other methods, such as but not limited to, computers, tissue culture, chromatography, spectometry, non-animal models, lower organisms, or dummies can be successfully substituted."

That particular resolution was referred to the Committee on Science and Technology, and no action has yet been scheduled.

In the House Committee on Agriculture, where jurisdiction over animal legislation has traditionally reposed, we now have before us H.R. 5808, which seeks to regulate principally the inter- and intrastate transportation of animals for research or other purposes. This act is designed to give the Secretary of Agriculture stronger regulatory control with respect to the conditions under which animals are shipped in interstate commerce, by common carrier and intermediate handlers, and to establish standards for such shipment.

I would expect that the Subcommittee on Livestock and Grains, which is now considering the bill, will report it favorably before too long, though this action may not coincide with the pending deadline for consideration by the Rules Committee. Thus, action in this session of Congress appears somewhat doubtful, though I would predict that successful completion of the legislation will occur later.

Congress has moved more vigorously in the field of animal protection in recent years than in the past, as is evidenced by passage of the Animal Welfare Act amendments of 1970, which strongly increased the regulatory authority over the care and handling of animals in, among other things, biomedical and other research areas.

Yet, as is sometimes typical of the Congress, it passes far-reaching laws without appropriating very substantial funds to enforce them.

The level of appropriations for the enforcement of animal welfare laws has been, in my judgment, pitifully inadequate. Some segments of the research community might welcome this fact, fearing that regulation might interfere with the success of important experiments. It seems to me, however, that sensible regulation both needs and requires a greater level of funding.

As to the overall attitude toward the substitution of *in vitro* systems for living animals in experimentation, I think it is fair to say that Congress has generally backed away from regulating the methods used in experiments involving living animals in the belief that such action might substantially threaten successful research. If one compares the amounts of money the Congress has appropriated for animal protection with the amounts appropriated for research in various areas, the differences are staggering. Often what determines the intent of Congress is not the authorizing of legislation, but how it divides the public purse.

As an institution, we are occasionally given to offering very far-reaching statements of high purpose voicing sensitive concerns, both for animals and for all areas of human welfare; but, when it comes to the appropriation of money, perhaps a deeper reaction is revealed. Woodrow Wilson once said, "Congress in session is Congress on exhibition; Congress in committee is Congress at work." One might develop a similar aphorism with respect to Congress appropriating and Congress legislating.

Of course, as you know, in the Congress the committees responsible for authorizing legislation do not appropriate the funds to implement these same programs. Instead, these are separate functions handled by different committees. As long as there is any substantial disagreement within the biomedical or research community concerning the necessity of using living animals for research, Congress will remain extremely reluctant to restrict research efforts using live animals other than in situations where experimentation involves unnecessary pain or discomfort.

In fact, most of the humane and welfare organizations in the United States have not attempted to cut off all or even substantial research with animals. Their purpose has been, rather, to encourage or require that, in the transporting or holding of animals, some minimum standards of proper comfort, feeding, climate, housing, and pain relief be followed where possible.

I do not really believe that the research community has much to fear from legislation preempting the course of research with living animals, unless and until the use of these *in vitro* systems has become so advanced that there is substantial agreement within the medical community that the large share of what is presently done with living animals can be reproduced or refined through the use of other methodologies.

As the newer techniques develop, there will probably be some pressure for their substitution in cases where they not only complement, but perhaps improve upon, experimentation with animals. On the other hand, the Congress has recently enacted new legislation, such as the Toxic Substances Act, that will, in all likelihood, require additional testing on living animals. Because these new acts and related regulations require that human beings be protected from certain harmful products in interstate commerce, I feel that their implementation will probably require some additional animal testing in order to assure that substances in commerce, such as food additives, cosmetics, and household products of various kinds, are not toxic or otherwise threatening to man.

Although this is a bit off the subject of our discussion, I suspect that we will also include in pending legislation dealing with the transportation of animals some effort to involve the federal government in prohibiting dog fighting or the fighting of any animals. The unacceptability of that grievously cruel practice leaves very little room for debate. Frankly, many members of Congress are now impatient with the Agriculture Committee for not reporting such a bill more quickly. As a matter of fact, I am continually importuned by colleagues from both cities and rural areas, as to when legislation can be brought out to end this practice.

There is always the danger that intense emotional reactions to some forms of biomedical research could cause a welling up of political activity resulting in the passage of ill-advised laws. One thing about the humane community in the United States, broadly speaking, is that it has mobilized enormous influence, cutting across all political parties, ages, regions, and economic considerations. Members of Congress, if they are good at anything, are good at sensing strong concerns held by their constituents. Whether we like it or not, this is a representative democracy. Many elected officials adhere to the principle that their sole function is to faithfully reflect the positions and attitudes of their constituents without judging whether they are right or wrong.

I think it is important to have a continuing exchange of ideas among humane societies, welfare groups, concerned citizens outside of the scientific community, and the biomedical research community itself.

I especially want to compliment the sponsors of this symposium. Furthermore, I think this is precisely what is needed, as we proceed with new developments in the various techniques of *in vitro* research, if we are to ensure that the various positions do not become so irretrievably polarized that the issue must be faced on very strongly political terms in legislation before the Congress.

I want to assure you that the Agriculture Committee has received excellent cooperation from the humane and welfare groups, as well as from the biomedical research community, in our attempts to frame legislation. I am sure that other committee chairmen dealing with this subject will encounter an equal readiness on the part of these groups to give counsel and to share experience.

From our standpoint, of course, such assistance is very much needed. All of us in the Congress are expected to act as specialists from time to time. Yet, because the majority of our congressional or parliamentary responsibilities most often require broad basic knowledge of a variety of subjects, we are increasingly conscious that we do not possess exact knowledge in any field and, therefore, must seek the help, counsel, and advice of specialists.

I would be glad to attempt to respond to any questions that you might have. This particular issue, with its important moral and philosophical implications, is presenting itself to the research and animal welfare communities in advance of any consideration by the Congress. Although it is therefore very difficult for me to assess what its attitude might be at this juncture, I shall be happy to attempt to answer any questions you may have.

ADDENDUM

Public Law 94-279, 94th Congress, April 22, 1976, amended the Animal Welfare Act of 1966 to increase the protection afforded animals in transit and to assure humane treatment of certain animals and for other purposes. Included in this legislation is authority and direction for the Secretary of Agriculture to prescribe rules relating to: health certification on animals being shipped, minimum age for animals being shipped, financial arrangements with carriers by person shipping animals COD, and prohibition of animal fighting ventures.

DISCUSSION

PRATT: What is the meaning of a concurrent resolution? How does it differ from a bill?

FOLEY: It really does not differ from a bill. It usually is used to express a sense of Congress in the form of a resolution rather than as a binding legislative requirement.

This concurrent resolution that has been introduced by Mr. Whitehurst does not require the cutoff of funds, nor does it require the federal government, in its various research agencies, to do anything. It merely expresses the opinion of the House and the Senate that this should be done. It is hortatory rather than binding, since it has no legal effect. In other words, no one can be taken into court or sued to force compliance. I suppose it can be regarded as a kind of Christmas card from the Congress, which, if passed, says, "This is how we feel at this particular season of opinion." Additionally, it may later serve as the basis for further legislative action, to use Mr. Whitehurst's resolution as an example, if it is passed. If Congress has enacted a concurrent resolution of this kind, perhaps its supporters may later argue that it should be implemented in some way. One method is an appropriation limitation, but there are other approaches. I personally have some objection to the use of appropriation bills for legislating basic policy.

In drafting a 1-year appropriation bill for any federal agency, be it the National Institutes of Health, the National Science Foundation, or whatever, you could simply add the phrase, "No funds under this act may be expended to support any animal research where alternatives. . . ."

Now, for the next fiscal year, that would result in a limitation on grants and other research-supporting programs. I will not bore you with the parliamentary details, which usually do not permit the legislation of policy in an appropriations bill.

Appropriations are to assess funds, to decide how they should be spent. The legislation of policy, on the other hand, is supposed to be handled by the so-called authorizing committees. Yet, there is a parliamentary maneuver called the limitation on appropriations, which says you cannot spend money under specified conditions.

The difficulty with this approach, as I see it, is that such a limitation is usually attached to an appropriations bill without allowing for prior consideration by the Appropriations Committee or any other committee. It may have immediate appeal to a certain number of people in the House, who would vote for it quickly without really taking the time to consider it in a mature fashion.

The other approach, of course, would be to introduce legislation requiring that, before awarding federal grants for research on animals, the Secretary or someone must first determine that there is no adequate *in vitro* alternative that can replace experimentation on live animals.

A British author, Mr. G. L. Gowans, wrote last year in the *British Medical Journal* that he felt such a restriction in British law would be unenforceable, since it would require an assessment of each proposed research project. Further, there is a serious problem if we develop standards that lead to mere paper assertions by the grantees.

How would we oversee or verify such assertions that there are no adequate substitutes? Who would make the final determination? It would be an extremely difficult regulation to enforce.

But at this stage, Mr. Whitehurst's concurrent resolution would not have any binding effect; if it were passed, though, it might lay the groundwork for future legislative action.

KURTZ: Perhaps one direction that the Congress might take in terms of a concurrent resolution is to encourage the regulatory agencies that already have established procedures for live animals, such as the Food and Drug Administration and the Environmental Protection Agency, who already have codified the procedures for use of experimental animals. Any exhortation should at least be directed toward the government agencies, that they should encourage and accept the use of *in vitro* models over live models where the former can be shown to be useful or predictive or are capable of substitution. Perhaps that might be a direction that Congress could approach, where they obviously cannot police every single research project that is going on in every medical center or drug company.

FOLEY: I think that that is a good suggestion. The first half of Mr. Whitehurst's current resolution is fine, indicating that the federal government ought to, in effect, encourage this approach by funding research for *in vitro* substitutes and encouraging that they be substituted where possible. The danger, as I see it, arises if we introduce a sharp requirement for cutting off federal funds. To make that effective, there would have to be a review procedure perhaps so elaborate that it would not be easy to enforce and might actually interfere with legitimate research on living animals.

The Congress is sincerely concerned that animals be treated humanely, whether they are destined for slaughter or for research. On the other hand, I think it is fair to say that there is no desire to interfere with the necessary and appropriate use of animals for research purposes. To find the balance between regulations that permit and encourage animal protection without limiting or interdicting necessary research is basically what the Congress will attempt. Certainly your suggestion of using the various agencies to encourage the development and use of alternatives, such as computer technology or the use of cell or tissue organ culture, is one that would find support in the Congress.

KOWRY: Would you tell me what the general feeling is in the Congress about various bills that have been introduced in the past. I do not know if there are any pending now with regard to reducing federal funds to public school systems that allow experimentation on live animals by kids.

Also, I am interested in the status of the more basic things to do with research, as, for example, the feelings of other humanitarians about the exercising of dogs.

FOLEY: Legislation reducing or cutting off funds to schools allowing experimentation on live animals has not been reported by the Congress. This issue, to my knowledge, has received little or no attention in recent years.

Traditionally, the protection of animals has been within the jurisdiction of the states, whose police are empowered to prevent cruelty. In more recent years, however, the situation has become more complicated due to the increased interstate movement of animals, which takes them beyond the jurisdiction of individual states, thereby requiring the involvement of the federal government.

The question of exercise of animals has been and remains controversial. Research institutions have claimed that it would be very expensive to provide extensive animal exercise facilities for laboratory animals. Though I am by no means an expert in this area, I think there is some feeling that the use of traditional cages is not the best way of holding animals, even for research. Perhaps new techniques ought to be examined for housing animals, such as those that have been developed for the holding of primates in various primate centers around the country.

At the moment, the exercise provision is contained in a proposed regulation. Moreover, the research community claims that with increased costs and with heavy demands, it simply does not have the money to develop animal runs and to provide sufficient personnel to handle the animals.

Discussions will no doubt continue on this point within the context of pending legislation.

TRUM: Congressman Foley, I call your attention to the fact that this symposium was conceived 3 years ago for the purpose you have so eloquently stated. That is, to bring to the attention of the reviewing committees and research personnel any new approaches that may be occurring or ways of improving their own procedures through methods not now being used.

Every research project sponsored by the federal government is reviewed in some manner, and each institution also initiates its own independent reviews. One of the questions for review should be: Are the most efficient of modern methods being used? In each case we believe they are, but we've heard we could do better. Perhaps we can be assured by this symposium.

I want to assure you that this symposium is an effort of the scientific community to promulgate the most modern methods available by calling attention to all the areas in which cells, models, and systems can be used and the future of animal use in biological research. Help from all areas of our society has been solicited, and facts and opinions are sincerely welcome.

FOLEY: I want to congratulate you and the committee for organizing this symposium. At the risk of being repetitious, I should like to make a small plea for understanding and guidance in behalf of my colleagues in the House and myself. Legislating in some of the more specialized areas can be very difficult for members of Congress, because, unfortunately, very few of us come from the scientific, technological, or research communities. Most of us are lawyers, businessmen, and farmers. Thus, scientific expertise has traditionally been lacking in the Congress, despite the fact that, in this age of rapid advancements in both science and technology, we are increasingly called upon to make informed decisions in highly specialized areas. In recognition of the fact that we needed the advice of experts, the Office of Technology Assessment was created; but we still seek the advice of people such as yourselves. Even with outside help, we occasionally find ourselves in a quandary when we turn to the specialists only to find that they are divided on the issue at hand. For then we must evaluate the merits of the various informed positions.

Obviously, gatherings of the type that bring us together today to discuss the current state of the scientific arts and the possibility of using new methods to eliminate experimentation on living animals can only be applauded. I am sure that the work of this symposium will be extremely valuable to legislators in making future decisions. I stress the word "future" because the issue we are discussing is one that very few members of Congress are now actively considering. Yet, if and when this topic becomes the focus of mutual concern to the public and the political and research communities, then Congress may well take up legislation. In that event, I would hope that its work would be conducted in a stimulating and objective mode, rather than in an effort to overly regulate or restrict developments that are proceeding peacefully and effectively without legislation.

I came to Congress 11 years ago imbued with the idea that legislation can cure all the evils of the world. Yet, I recall more and more the wisdom of Kierkegaard, who said, "With what little wisdom is the world governed." It can be said that it was not governed very well then, and perhaps very little has changed.

To legislate in an effort to correct obvious abuses or to deal with problems that require federal regulation is one thing. In the sphere of animal protection and welfare, the best and surest approach, in my view, would be a cooperative effort on the part of the research community, the humane and animal welfare organizations, and concerned citizens; for I know that the concern for animals is not limited to animal welfare organizations. It exists in the scientific community as well.

I would hope, therefore, that organizations engaged in efforts such as this symposium would produce whatever progress is necessary in the field without the imposition of overly restrictive legislation.

I particularly want to avoid passing legislation that we then fail to implement and fund. More and more, I think that either we must limit what we do or back up our actions more adequately with real resources. Thus far, we have tended in many areas to promise more than we have been able to deliver.

Having said that, I think the legislation that has been passed has certainly not been overly restrictive. In fact, there are still many areas of animal welfare that I feel have not been properly addressed.

Obvious and egregious cruelty to animals continues in such things as dog fighting, the handling of animals in transportation, and particularly in the handling of animals for slaughter. These are among the problems that state governments are simply not addressing themselves to adequately, and I think the Congress will have to act in these areas.

T. C. HSU

Cells in Culture in Biology, Medicine, and Public Health

For several decades, tissue culture was primarily the tool of histologists and nutritionists. The system of culturing tissues in the early years was necessarily crude, and the ingredients of the growth medium were usually natural products, such as chick plasma, embryonic extract, and serum plus salt solutions. Most of the observations were limited to cells in primary cultures, and, if continual cultivations of cells were performed, the cells were transferred *en masse* as if they were in tissue.

Slowly, cell culture, instead of tissue culture, became successful, and monolayer cell lines were established. This allowed profitable research into the nutritional requirements of cells in culture, and many supplementary nutrient formulas were devised. Finally, the supplements replaced most of the natural nutrient ingredients in the growth media, except serum. Although a number of cell lines have now been adapted to grow in completely synthetic media, this system is not yet popular, because the cultures need meticulous care and expert handling. Furthermore, to grow cells in serum-free media requires long periods of adaptation, and cells capable of growing in such media are usually aneuploid in their chromosome constitution. Therefore, it is conceivable that drastic changes in the genetic makeup of these cells have taken place, and the cells are not suitable for experiments requiring "normal" cells. Currently, most investigations using cell cultures still employ serum supplement. Other than possibly introducing mycoplasma and bacteriophage contaminations, a small amount of serum that contains unknown nutrient factors does not hinder the validity and efficiency of most investigations.

The finding by John Enders that mammalian viruses can grow in cultured mammalian cells was probably the most significant single event that promoted cells in culture as a popular tool for biomedical research as well as a stimulant of multimillion-dollar business. Without cell culture, vaccines for poliomyelitis might not have been available. In the early phases of viral studies, virologists simply used mammalian cells as a "growth medium" for animal viruses, and few endeavored to learn the biology and the chemistry of the host cells. However, researchers from other disciplines began to utilize mammalian cell cultures as materials for research. The results expanded our knowledge of cell structure, cell physiology, cell genetics, pathogen–host cell relationships, and others. In many cases, one achievement influenced another.

One of the very significant contributions made by using cell cultures was the analysis of biochemical events of the cell cycle. Sophisticated techniques were developed to synchronize cells with respect to the cell cycle, thus facilitating chemical and physiological determinations of individual and collective events that could not have been achieved by using intact tissues. Cell cultures allow the pulse exposure of cells to any physical or chemical agent for a given period of time (as short as less than a minute). Such precise experiments cannot be performed with cells *in vivo*, because a drug cannot be removed once it is injected into an animal. Studies on the cell cycle have myriad applications, including cancer chemotherapy.

Another important technological advance was the invention of a relatively simple method for obtaining colonies (clones) derived from single cells. This procedure is extremely useful in experiments relating to somatic cell genetics. It provides a quantitative approach for estimating cell survival and reproductive capability. Exposure of cells to a number of detrimental agents, e.g., ionizing radiation, does not immediately kill the cells, but it prevents the cells from reproducing progeny. Cloning gives a fair estimate of the percentage of cells surviving to reproduce under a given condition. Thus experiments with mammalian cells can be handled in a way similar to experiments using microorganisms. Moreover, colony formation permits investigators to isolate single-cell derivatives for cell cultures with genetic purity—a must not only for genetic analysis, but also for other experimentation as well.

In the 1950's, when cell-culture systems began to gain popularity in biomedical research, investigators used several established cell lines, such as HeLa, a human carcinoma, the mouse L cells, and others, as their primary source of material. Unfortunately, all of these permanent cell lines were abnormal in many respects, particularly in their genetic makeup. None had a diploid chromosome constitution, and the chromosome composition varied from cell to cell, giving heterogeneous cell populations. In many studies, e.g., in producing viral vaccine for human use, a uniform, diploid cell population is of prime importance. Since cancer cells are known to possess abnormal chromosome constitutions, any cell line that is derived from normal tissues but has chromosome changes is suspected of neoplastic transformation. It is a serious concern that these cells may harbor latent oncogenic viruses, and the resulting vaccines may accidently be contaminated with oncogenic viruses or may have picked up from the host cells genes that are capable of inducing cancer. In most commercial production of polio vaccine, primary or early subcultures of monkey kidney cells were used. This method has several undesirable factors:

1. One does not know whether the animals carry latent viruses that may cause severe cellular damage after *in vitro* cultivation.
2. Since a new monkey is used every time, it is impossible to set a quality standard.
3. Many animals are killed just for a pair of kidneys. Such a practice necessitates importation of thousands of rhesus monkeys and African green monkeys annually, thereby depleting the supply of the animals, changing the jungle ecology, and endangering wildlife.

In addition to these considerations, many biomedical experiments require a large supply of relatively homogeneous diploid cells or at least cells of the same genetic compositions as those of the donors. Attempts have been made to cultivate cells of various animals, particularly human from biopsy specimens, to determine the feasibility of procuring long-term cell lines without detectable changes of the original genetic makeup.

Such attempts have produced a partial success. Many laboratories found that, with proper care and proper routine procedures, it was not difficult to initiate cell cultures from small biopsy specimens from any animal, including human subjects. The cells can be propagated with vigorous growth, giving rise to an astronomical number of cells for many experimental or production purposes. The cells maintain their diploid constitution with very little change. However, the cell lines so established, particularly those of human origin, do not perpetuate indefinitely. They slowly lose their growth potential, and, after 40–50 passages, they succumb. Many investigators consider this to be a phenomenon of cell senescence. Nevertheless, within the life span of a diploid line, one can obtain a large quantity of homogeneous cells; and thanks to the advances in cryobiology, one can preserve cells of early passages for future use. Therefore, the senescence phenomenon is not of great concern for practical purposes. The well-known WI-38 human cell line established by Leonard Hayflick is a good example. All pharmaceutical manufacturers use this line for production and testing.

During recent years, practically every branch of biomedical science has utilized cell cultures in some experiments. Human cytogenetics, for example, could not have been started without cell-culture techniques. Most hospitals have set up cytogenetic laboratories for routine services. Somatic cell genetics, which has yielded some important information in basic genetics and medical genetics, relies on cells in culture for critical experiments. Many molecular biologists also have begun to use cell-culture systems for their work.

Cell cultures are now being used in another research area of national concern, the testing of environmental mutagens. With a constant supply of homogeneous material, with the ease to expose and to interrupt or stop the exposure of the cells to mutagens or possible mutagens, with many results that can be quantified (survival rate, mutation rate, structural changes, chromosome aberrations), mammalian cells offer an excellent system for testing and monitoring mutagenic activities of a given agent. The data should complement that which can be obtained in microorganisms and lower animals such as the fruit fly *Drosophila*.

We may summarize the advantages of cell-culture systems as follows:

1. They provide a continuous supply of homogeneous cellular material for biological and biochemical experiments as well as for practical use in medical and public-health work.
2. The cells *in vitro* can be advantageously manipulated in many ways. This cannot be done with cells *in vivo*.
3. They can be stored in a deep-frozen state without changing their growth rate and genetic composition, and they can be revived at will.
4. Using cell cultures is more economical than rearing animals and performing experiments with intact animals.
5. They save lives of animals.

Without question cells in culture will be utilized more extensively in biomedical research in the future, and new methods will be devised to increase the applicability and accuracy of the system. However, it must be emphasized that cell cultures, which lack the cell–cell interrelationships that exist in tissues, should be regarded as only one type of good research technique. Even organ culture cannot replace the necessity of experimentation with intact animals or, in some cases, human subjects. Information obtained from cells, e.g., chemotherapy, must be carefully tested in the animal rooms and finally clinics, because cell cultures deal with individual cells, not tissues, organs, or the body as a whole. Nevertheless, *in vitro* systems offer endless possibilities for preliminary (and in many cases, final) information, and efforts should be made to fully utilize and improve them for effective information-gathering, for economy, and for humanity.

MARY DAWSON

In Vitro Systems in Basic Biomedical Research

> "Progress has become a race—and only man has not entered for it. Our sewing-machines are better than they used to be, our aeroplanes fly faster and have greater expectation of arriving, our medicines are more deadly year by year; but man, dear whimsical man, shows no improvement whatsoever. And on the whole," said the Count, "I dare say that is a good thing. Try to imagine a human being, emulous of the machines, who had become perfect in all his parts and scientifically efficient. How horrible he would be!
>
> From *Private Angelo* by Eric Linklater with permission from ADPeters & Co Ltd., Writers' Agents, London.

HISTORICAL BACKGROUND

Tissue-culture originated towards the end of the last century from experimental embryology. Embryologists then, as now, were intrigued with the orderliness of development, and they sought factors governing this first of all by transplanting fragments of one embryo to different places in that embryo, then by transplanting fragments from one embryo to another of the same or different age, and finally by trying to grow fragments out of living systems altogether and *in vitro*. Accounts of these early experiments in culture, together with later work arising from them, are to be found in Kopac (91), *History of Strangeways Research Laboratories* (83), Fell (60), Penso and Balducci (114), and Paul (113).

The object of the early work was to gain knowledge of the cells, tissues, or organs themselves in a more precise, controllable, and observable system than could be achieved *in vivo*. However, the factor that disheartened early workers was the high incidence of loss of cultures by bacterial contamination. It is interesting to quote Ross Harrison (81) on this topic:

> . . . it was found that bacteria quickly invaded the preparations, often destroying them as soon as the second day after implantation. . . . After experimenting a little with antiseptics such as thymol and acetone-chloroform, it became apparent that satisfactory preparations could not be obtained except by working aseptically. . . . Several epidemics of mould (Penicillium) were encountered, but this too grew slowly, usually from a single spore or two, and as it does not seem to kill the embryonic cells, it interferes but little with the observations.

It is a pity that he did not observe that the penicillium-infected cell cultures rarely succumbed to bacterial infection or he would have "discovered penicillin" 19 years in advance of Fleming (64). It must be one of the nearest misses of a valuable discovery ever to be reported.

However, with the advent of penicillin and other antibiotics, it became customary to add these to culture media, and this usage, probably more than anything else, led to the great, and still growing, use of tissue culture. The commercial availability of cells of guaranteed identity, and of media guaranteed to support their growth, means that any normally equipped biological laboratory can now undertake tissue-culture experiments. The flood of papers indicates that more and more groups of workers see something in the technique relevant to the questions they are asking. The original question asked by the early embryologists was, "What starts a given group of cells proliferating, differentiating and moving at a given time, and stops them doing it at a given time?" The question has not yet been wholly answered, and it is now of course recognized that it is relevant not only to embryology, but also to wound healing, teratology (drug-induced disease), and to cancer. A recent review (34) discusses current thinking on cancer, differentiation and embryonic antigens, and indicates the various unresolved questions.

CURRENT TECHNIQUES IN TISSUE-CULTURE

The term *tissue-culture* is generally used to cover culture of cells, tissues, and organs of various animal (vertebrate and invertebrate) and plant species. I shall speak mainly about vertebrate cultures and mainly of mammalian cells within that subdivision, as these are the cultures with greatest relevance to biomedical research.

Cell Culture

This means the growth of usually only one cell type at a time. The cells may be derived directly from an *in vivo* source (primary explants), may have been subcultured a few times from this as it grew (diploid cells), or they may be of several years standing in culture (heteroploid cells), i.e., with altered chromosome numbers. There have been many papers on suitable media for growth, but generally only the heteroploid cells will grow in chemically defined media. Even "chemically defined" is very complex, the media containing many inorganic ions, amino acids and vitamins, and glucose. For primary explants and diploid cells, serum is usually required, except for short-term growth such as in viral vaccine production. The cells usually grow as a monolayer on the bottom of bottles or flasks laid flat in an incubator and held at a temperature appropriate to the species.

There are various devices for larger-scale cultivation of cells. Some cells, again usually heteroploid, will grow in suspension. Microbiological fermenters have been adapted for this. Anchorage-dependent cells, mostly diploid, have been grown on spirally wound plastic sheets in sterilizable containers, to obtain large amounts.

At the other end of the scale, cells have been "cloned," i.e., colonies produced of cells all descended from single cells, to obtain greater uniformity of material (56,65,100,105,123,124,139,185).

Also small numbers of cells have been grown in various designs of optically good "perfusion chamber," so that they can be examined live on a phase-contrast microscope and the effect on them watched while various drugs are added and removed. Such apparatus, or components of it, has been described by De Haan (53), Schade (141), Tinsley (157), Richards (128), Hughes and Swann (88), Fell and Hughes (62), Hughes (85,86), Mackaness (95), Christiansen *et al.* (30), Stockinger (150), Schalow (142), Buchsbaum and Kuntz (21), Frederic (68), Pomerat *et al.* (120), Rose (133), Schwöbel (144), Barer (9), Harris (78), Michaelis (97), Richter and Woodward (129), Von Stosch (173), Barski and Robineaux (12), Bessis (17), Pulvertaft *et al.* (125), Robineaux (132), Michel (98), Wolfgram and Rose (183), Constable and Moffat (36), Toy and Bardawil (163), Biggers *et al.* (19), Cruickshank *et al.* (42), Schiemer (143), Sharp (146), Wischnitzer (182), Freund (71), Hughes and Cardew (87), Lawson (92), Liss (94), Orsi (109), Pruniéras and Chardonnet (122), Pybus (126), Roberts and Trevan (131), Sykes and Moore (153), Trevan and Roberts (164), Anonymous (6), Brooijmans and Verbeek (20), Mosolov (104), Dawson (46), Rose (134), Dawson *et* *al.* (48–50), Verbeek (171), Dawson and Matthews (51), and Paul (113). The length of this list indicates the value to be obtained from direct observation of unstained, living cells. An apparatus for this purpose, designed for time-lapse cinemicrography, is shown in Figure 1. Time-lapse cinemicrography saves one from spending time actually watching the cells and condenses the time required for observation by projecting at a speed greater than the filming speed. A perfusion chamber is shown in detail in Figure 2.

Tissue and Organ Culture

The object of this type of culture is to keep the cultured material as near as possible to its *in vivo* appearance and function and at the same time have improved access to it. This type of culture is not so widely practiced, but nevertheless it has several famous exponents, notably Dame Honor Fell in England, Professor Gaillard in the Netherlands, and Professor and Mme. Wolff in France. Useful comprehensive accounts of organ culture are to be found in Dawe (45) and Balls (7). It is of interest to record a return to the technique, principally in cancer research, almost a century after the idea was propounded (16). The organs are usually embryonic, partly because it is often desired to

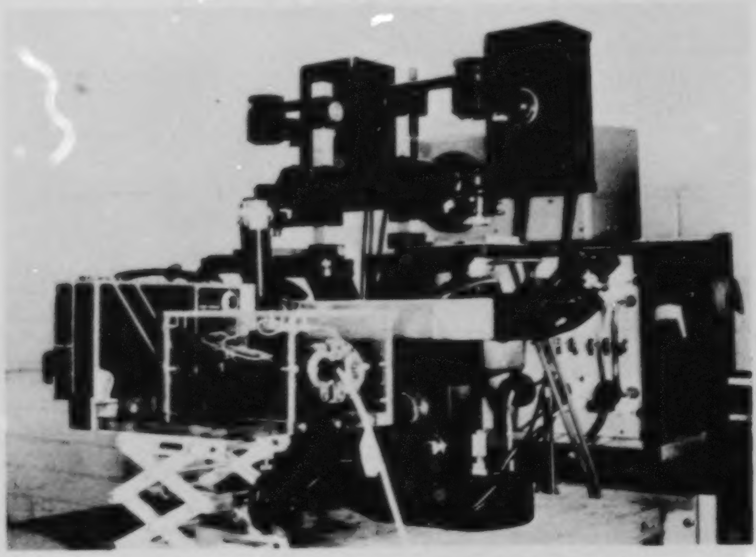

FIGURE 1 Apparatus for time-lapse cinemicrography.

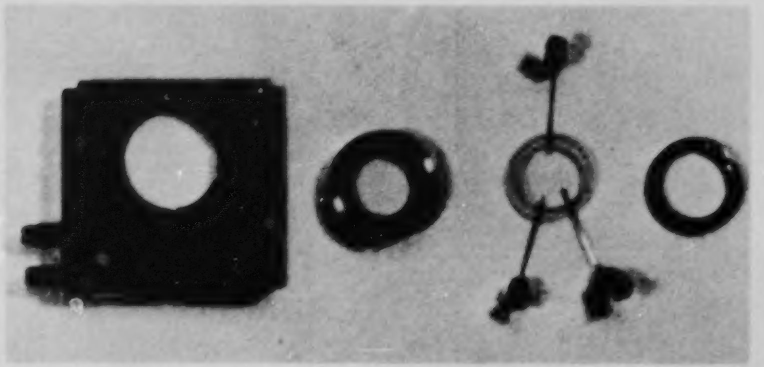

FIGURE 2 Perfusion chamber showing brass water-jacket for temperature control and titanium chamber to contain cells between two coverslips that are separated by a silicon rubber gasket.

study these and partly because they grow well and are small enough to allow nutrient and gaseous exchange right to their centers, whereas larger fragments tend to necrose in the middle. They are usually grown floating on rafts or lying on supports with liquid or semisolid nutriment touching their undersides while the upper surface is exposed to a suitable gaseous atmosphere. The container may have a reservoir of this.

These then are the two main subdivisions of tissue-culture as currently practiced, and, within both, almost any part of the animal may be grown or at least induced to survive for an experimentally useful time.

APPLICATIONS CURRENTLY MADE OF TISSUE-CULTURE TO BIOMEDICAL RESEARCH

Tissue-culture had not been long practiced until it was recognized that it had valuable applications other than adding to our knowledge of the biology, biochemistry, or pathology of the cells themselves. The seven applications mentioned here are by no means unrelated, and each could itself be the subject of a lengthy review.

Virology

Tissue-cultures have been used to grow viruses for many years (149). Tissue-cultures are very suitable for this, as viruses are intracellular parasites and are more easily controlled in cells grown in closed bottles than, for example, in animals that might be excreting them. An account of the various virological uses of cell cultures is to be found in the paper by Enders (57). Studies can be made of the viruses themselves, for taxonomic purposes or to study their methods of attachment, penetration, and multiplication in cells; the combination of cell and virus can be used to study interferon inducers and inhibitors and antiviral chemicals; the host cells can be studied for factors leading to resistance. In addition, cell cultures are widely used to manufacture and assay viral vaccines (115). Some of the earlier tissue-culture viral vaccines were unsatisfactory in respect of low immunogenicity and contamination by other, previously unheard of, viruses. However, manufacturers take great precautions with respect to viral and host cell identity, purity, and stability and viral inactivation if relevant (152,177). Indeed, tissue-culture's role in lessening viral disease has been very notable. It is largely responsible for the decline in poliomyelitis and yellow fever. The three fields of virological research most prolific in publication at present seem to be firstly that of uncovering new viruses, secondly that of using virus to "transform" cells from a normal to an approximately malignant condition, and thirdly that of using viruses to fuse cells for genetic studies. These latter two topics are mentioned again under the sections on cancer and genetics. Cultures of arthropod cells are of use in isolating some viruses (127).

Cancer

This was one of the earliest applications of tissue-culture (172). It is impossible nowadays to imagine cancer research without tissue-culture. There is an almost intractable mass of papers on differences between normal and malignant cells and almost equally intractable lists of substances that have been screened in tissue-culture in the search for anticancer drugs. This aspect will be mentioned under pharmacology. The normal/malignant differences can probably best be tackled by computer, so numerous are the variables among the systems used to study both the differences and the causes of onset of the change from normal to malignant. A recent excellent account of the pros and cons of tissue-cultures and animals in cancer research has been given by Franks (67). The main advantages are those applying to all tissue-culture experiments, but an important limitation is that the common tumors arise from differentiated cells and it is difficult to keep cells differentiated in culture. The immune relationship of a tumor to its host is also of paramount importance for its regression. Also, the real criterion of malignancy is tumor production on replanting into an animal, and cultured cells may increase or decrease their capacity to do this or develop for no known reason such a capacity where none originally existed. This is called "spontaneous" transformation, as distinct from transformation deliberately induced by viruses or chemicals.

An extensive account of the differences between normal and malignant cells can be found in a Ciba Foundation symposium report (184), and many papers on this topic can be found also in a publication edited by Hall (76). A full account is given of the cancer cell *in vitro* by Ambrose *et al.* (4). Another useful collection of papers on this and other topics is that assembled by Pollack (117). The references listed in the remainder of this section are by no means comprehensive, as this is beyond the scope of a review paper covering other topics besides culture of cancer cells. The papers cited do, however, contain references to the earlier work that led up to them.

Since about 1970 there have appeared very many papers on contact inhibition of growth or movement (a property of "normal," cultured cells), on the "transformation" of these cells by viruses or chemicals, and on biochemical and surface differences between resting and cycling cells. Contact inhibition is widely observed in cultures and is usually taken as an indication to split the culture into two or more so that growth may resume. It is thus not a permanent change. Contact inhibition of a nonreciprocal type has been studied by cultivating different cell types together, so that they may interact as they do *in vivo* and give some insight into tumor invasiveness (175). Contact inhibition was related to cAMP and testosterone by Hsie and Puck (84), who related these substances in turn to the promotion of microtubule organization. It was related also to pH by Ceccarini and Eagle (28) (but this was regarded as but one factor in the system), and it has been related also to enzymes transferring galactose (135). An effect of contact was found to be decreased uridine uptake (188), but whether it was a direct effect or occurred via decreased membrane mobility was unresolved.

Altered proteolytic activity of "normal" and "transformed" or "malignant" cells has been the subject of much recent study. Burger (22) reported that the normal mitotic cell temporarily had a surface like a transformed cell and that proteolytic enzymes could induce temporary escape of normal cells from density-dependent inhibition of growth. The topic was taken further by Unkeless *et al.* (167) and Ossowski *et al.* (110) who found that tumor and transformed cells secreted a substance that, together with a serum factor, led to a very specific fibrinolytic activity. The serum of tumor-bearing animals contained protease inhibitors of the fibrinolysis, not detectable in controls, and the increased fibrinolysis seemed not to be due to changed amounts of lysosomal enzymes and did not follow infection with a nontransforming virus. This work arose from the authors' reexamining work of 50 years earlier, concerning tumor-cell destruction of fibrin clots, these being the supports used for cultured cells at that time. It would be interesting to know more about the mechanism of removal of fibrin clots *in vivo*—in relation to immune surveillance—and even to consider whether in old age one has a choice of either tumor cells or clots. This is, of course, purely speculative at the moment. Darżynkiewicz and Arnason (44) considered that previous hypotheses on the relation of protease inhibitors to unrestricted growth were perhaps too general and that their molecular weight and solubility might have to be taken into account. Collard and Smets (35) in the same year also observed differences in this respect among different protease inhibitors. A recent paper of Teng and Chen (154) discussed the need to reassess the role of some proteins removed by trypsin that were previously thought relevant to growth control, and Chen and Buchanan (29) reported a new type of proteolytic activity in virus-transformed cells. Thus it can be seen that these enzyme differences are considered of fundamental importance to elucidating the differences between normal and malignant cells.

The ability of some viruses to "transform" cells and the precise relationship of transformation to tumorigenesis is another topic currently interesting many groups of workers. It appears (23,151) that any one transforming virus is not the best one for all cell types. Reports on transformation always now detail both cell and virus type used.

Other distinctions found between normal and transformed or malignant cells include: changed glucose utilization (4), loss of anchorage dependence (69,96,116), rearrangement of intramembrane particles as seen by freeze-fracture electron microscopy (72,162), altered organization of actin subcortical sheaths (118) and the part of actin and myosin in cell movements (119), reduced membrane fluidity as indicated by rearrangement of binding sites of concanavalin A and other substances (14,147,186), and reduction in cAMP levels (33,101,137,158). The work of Willingham and Pastan (180) brought together these last two considerations in a hypothesis relating the greater agglutinability of transformed cells by concanavalin A to their having more microvilli, which was associated with their having less cAMP. Continuation of listing recorded differences shows that the following have also been studied: the relation of membrane potential to mitosis (138), the inverse correlation of prostaglandin E production with growth rate independently of its role via cAMP (156), the occurrence in transformed cells of a double-stranded RNA (54), the presence in malignant cells of membrane-associated DNA (2), the fact that chromosome 7 had a gene or genes coding for transformation factor (40,41), and the hypothesis that circulating DNA might play a part in oncogenesis (111). Recently, physical measurements by nuclear magnetic resonance have shown cancerous tissue to have less ordered water than normal (3,43).

It will be seen that cAMP has cropped up in connection with various changes between normal and malignant cells. An interesting unifying hypothesis, associating many such changes with lowered cAMP, was put forward by Pastan and Johnson (112). They considered that altered cell membrane adenylate cyclase might be primarily responsible for the differences.

It is thus clear that, although the only unquestionable criterion of malignancy is still the formation and progressive growth of tumors *in vivo*, there have been many valuable advances in recent years ascribable to the use of cultured cells as adjuncts to animal experiments and clinical observations.

Other Pathologic States

Although much more work has been carried out in virology and cancer than in relation to other pathologic states, there have been experiments of fundamental interest and clinical application there too. For example, tissue-culture has led to further understanding of gout, as indicated in the various works cited by Dawson (47) and the more recent work of Bandmann *et al.* (8). These works investigated the mode of action of colchicine and related antitubulins and the part microtubules play in directed locomotion and in phagocytosis of, *inter alia*, urate crystals. The primary interest of most tissue culturists in colchicine is as an antimitotic to accumulate cells in metaphase for chromosome examination—hence the large amount of work on colchicine and related substances available for application to the study of isolated stages of the disease.

Rheumatism, too, has been studied from two main directions *in vitro*. The works of Fell and associates (61) on articular organ culture were designed to shed light on factors concerned in the breakdown of articular cartilage in rheumatoid arthritis with special reference to autoantibodies. The authors were careful to point out that, although indications of a role were seen from the method, nevertheless an organ culture differs from an *in vivo* joint in lacking blood circulation.

Therefore, the complete answer to the pathogenesis of rheumatoid arthritis cannot be reached by *in vitro* work. Cartilage organ culture experiments have been carried out too by Jacoby (90) to compare normal and osteoarthritic material. The other aspect of *in vitro* work in relation to rheumatism is in studying the mode of action of antiinflammatory drugs. These will be mentioned in the section on pharmacology.

Psoriasis has been a puzzling condition, and obviously the accessibility of skin samples has led to their being grown *in vitro* to study this condition (25). Earlier, extensively mathematical, work related to this was carried out by Ryan *et al.* (136). More recently the all-pervading cAMP has been implicated by Vorhees *et al.* (174). These authors consider that a decrease in morphogenetic cAMP and rise in proliferative effector cGMP may be involved, but the question of whether the decrease is brought about by decreased synthesis, increased degradation, or loss by diffusion remains to be answered. The experiments of Harper *et al.* (77), however, using compounds that lowered cAMP, found no qualitative or quantitative difference in this respect between cultured keratinocytes from normal and from psoriatic skin. The answer to the puzzle is thus still elusive.

Genetics

Tissue-culture is an obvious tool to use in studies of mammalian and other cell genetics. There are many papers on the karyotypes of various species, including the famous but very modestly written report of Tjio and Levan (159) on the chromosome number in man. The instability of chromosomes in long-term cultured cells, and factors affecting it, is important in viral-vaccine production. Recent work on fusion of cultured cells has led to great advances in gene mapping with applications to genetic diseases associated with abnormalities, to the discovery of carcinogens and mutagens, and to the relatively new science of pharmacogenetics—the abnormal metabolism of some drugs in patients with various genetic abnormalities.

Early cell-aggregation experiments leading to later fusion experiments were performed by Moscona (102,103). Cell fusion was described also by Barski *et al.* (13), Barski (10), Ephrussi and Sorieul (58,59), and Barksi and Belehradek (11). Much recent fusion work has been carried out by Harris (79). Fusion was found to be aided by the presence of Sendai virus, first of all "live" (108) and then inactivated (80). More recently a nonviral fusogen, glyceryl monooleate, has been described, which may lead to the discovery of other chemically defined fusogens that would be preferable to the viral methods used hitherto

(39). Results from cell-fusion experiments are likely to have a tremendous impact on biomedical knowledge.

Carcinogenesis and Mutagenesis

In the development of new drugs there is probably greater need for a test for these characteristics than for any other test. This appears to be the consensus of opinion of the manufacturing firms who are the source of most new drugs, and it has been the subject of a recent World Health Organization (WHO) report (178). However, this report makes no specific mention of tissue-culture tests, nor does the First Report of the Expert Committee on Drug Toxicity (the Hennessey report) (63) or the Notes on Applications for Product Licences published by the Department of Health and Social Security from 1971 onwards (107). However, a more recent WHO report (179) does include such mention, and an updated version of the Hennessey report is due to appear shortly. It appears at present, from consideration of as many as possible of the available publications on carcinogens and mutagens, that no one test, *in vitro* or *in vivo*, will suffice, but rather the sum of evidence from several will be required. It is premature to speculate on the final form of or requirements for such tests.

The association between mutagenesis and carcinogenesis is not clear, but the 1974 WHO report regards it as sufficient to justify the use of mutagenesis tests as prescreens for possible carcinogens. Clarifying the association between mutagenesis and carcinogenesis is not improved by trying to compare mutagenesis findings, which mostly concern microorganisms, with carcinogenesis findings, which mostly concern rodents *in vivo*. The 1975 WHO report suggests using as an *in vitro* test system some of the several cell lines now available with suitable phenotypic markers and methods of isolating mutants. Trosko and Yager (165) suggest a sensitive and rapid method to measure DNA repair after carcinogen-induced damage, using various deficient media.

The study of chromosome aberrations has been greatly aided by the apparatus and methods of Caspersson and co-workers (27). These automated methods are under continuing development.

Aging

While this may not be regarded as a pathological condition, it is an inescapable condition, the details of the changes in which can be studied *in vitro*. Much of the work has stemmed from the observation that, whereas most diploid mammalian cells in culture had a limited life span of about 3 months, a minority became altered and immortal. There are, of course, many widely used established cell lines that have outlived their original source. The idea was put forward by Hayflick and Moorhead (82) that cultured diploid cells had a fixed number of possible generations *in vitro*, but Gey *et al.* (73) regarded their life as limited more by calendar time than generation number, basing their opinion on greatly increased survival time on collagen as opposed to glass as a substrate.

Absher *et al.* (1) used time-lapse cinemicrography in a painstaking study of the increase of generation time, and increased variation in it, of cultured WI-38 cells—the cell type hitherto commonly used in viral-vaccine production. While this is useful in providing information on cultured cells, the relation to *in vivo* cells is far from obvious. However, study of this was not the object of this work. The relation of life span of the species to life span of cells explanted from it was studied by Goldstein (74) using tortoise and human cells. The former lived longer in both cases. Also cells from young tortoises lived longer in culture than those from older ones, a common observation, though not an invariable one, with human material and material from other sources also (148). It would be interesting, though not easy, to set up models for reduced immunocompetence with age, investigating cell-surface antigen changes. However, aging is a field of work where there are obvious pitfalls in extrapolation from *in vitro* to *in vivo*, although scavenging *in vitro* has been convincingly demonstrated by time-lapse filming (personal demonstration, C. E. Lumsden, late of the University of Leeds, England). There have also been tissue-culture experiments on procaine and aging, but the results were variable. We have not observed prolonged survival on using procaine on cultures for other purposes.

Cellular Pharmacology

It is probably in this field that there is greatest scope for *in vitro* systems in biomedical research. They can be used in screening potential drugs for toxicity and for effects and side effects and for routinely assaying successive batches of manufactured drugs not amenable to physical, chemical, or immunological assay. Most countries have extensive and growing safety regulations with which manufacturers have to comply before a product can be marketed. These in general are effective, but the cost is increasing, especially in countries who are late starters in the field and tend to add together every other country's requirements. Manufacturers, therefore, have a financial incentive to look for replacement tests for their drugs. However, before any such test could be recognized, it would need to be very clearly shown, by carefully planned experiments with the existing animal tests being done as controls, that the new test, in addition to being cheaper, measured the same thing and measured it at least as well. There are several advantages to be gained from use of these systems. At the same time the limitations must be kept in mind.

ADVANTAGES AND LIMITATIONS OF TISSUE-CULTURE COMPARED WITH WHOLE-ANIMAL TESTING

Advantages

1. Errors in the effect of drugs due to species differences are eliminated since human material can be used. This material can be in the form of primary explants from aborted fetuses (but not in every country), material removed at surgery, donated material, remnants from skin grafts, or commercially available established material. All these materials, however, generally require animal serum to support their growth. Human serum may be used—for example prepared from the aliquot of blood donations used for grouping and the various other safety tests—but is generally variable, even if pooled, and far from likely to be free from medicaments or even alcohol, nicotine, or caffeine. That species difference is very important needs no emphasizing since the thalidomide findings, and even very closely related species can respond very differently to a drug, for example, chicken, pheasant, pigeon, and mallard to antimycin A (130). It is therefore surprising to find the continued use of cultures from other animals in testing drugs that are to be used in humans. There have even been recent papers recommending amphibian material on the grounds of its being longer lived (7). However, if a direct correlation were shown in the case of any one drug, there would be no objection on these grounds to the use of cells from other species in routine assays of, or tests on, that particular drug. Their use, however, is undesirable as well as illogical in either screening potential drugs for effects or side effects or in cancer or aging research relating to humans.

Differences in results obtained from the use of human and other cells have recently been reported by Harper *et al.* (77), using histamine on human and mouse cells, and by Lind *et al.* (93), using human- and mouse-marrow cultures for assaying granulopoietic stimulating factors.

2. A drug can be tested against a range of cell types—e.g., epithelial

cells, fibroblasts, blood elements, nerve cells, heart cells—both healthy and diseased and both embryonic and postnatal—all in the absence of detoxifying systems such as liver. The drug's toxicity to, and mode of action on, each type can be investigated, since one knows the exact concentration of drug bathing the cells *in vitro* and the duration of contact. The drug can be placed inside the cell by micropipette if so wished.

3. The effect can generally be directly observed or measured biochemically.

4. Since the application is direct, the effect often takes place rapidly.

5. Generally less drug is required for cell-culture experiments than animal experiments—an advantage if it is expensive or has been synthesized or extracted from a natural source only in small amount.

6. A statistically satisfactory number of replicates and control experiments can be set up more readily in culture than when using whole animals.

7. Cells can be kept deep-frozen in liquid nitrogen. There are programmed freezers commercially available that freeze at a rate leading to maximum viability on reconstitution. The enables identical material to be used for later experiments. There is always a risk of biochemical, chromosome, or other alteration to cells kept growing for a long time in culture.

8. Cell cultures are not bulky or subject to quarantine regulations and therefore can be flown to any country. The advantages of comparing drugs on the same material are obvious.

9. Radioactive materials can be used in cultures where they could not be used in humans.

10. As regards cost, it is certainly not cheap to set up a tissue-culture laboratory; but, if animal tests are performed, light and electron microscopes are required for histology anyhow, and the continuing costs of buying, housing, feeding, and looking after animals is greater.

Limitations

In vitro experiments can detect only a drug that acts directly in or on a cell. Thus *in vitro* experiments cannot replace animal experiments of certain types, for example:

1. For studying drug effects in any systems that are multistage, such as testing for teratogenicity or in fertility or blood-clotting studies.
2. For studying behavioral effects.

3. For detecting, studying, or measuring a drug that is metabolized into a more or a less active form or a more or a less toxic form.

There have, however, been tissue-culture experiments of these types, but they must be taken as studies of only one stage isolated from a complex system and not as substitutes for animal tests.

One further practical point is that in tissue-culture tests one must be sure that in the course of the test the drug is not decomposed by being kept in solution at 37°C for the duration of the test. This point is not always taken into account.

The conclusion to be drawn from considering the advantages and limitations is that tissue-culture certainly can not replace all types of animal experiments, but can frequently either add to the knowledge gained from them or provide more accurate knowledge.

STAGES IN THE DEVELOPMENT OF A DRUG WHERE TISSUE-CULTURE MAY BE OF USE

There are various stages in the development of a drug where additional information may be gained from *in vitro* tests. The normal steps in a drug's development, though variable of course for each drug, may be listed as in Table 1, reproduced here with the permission of the World Health Organization's Division of Publication and Translation, Geneva.

Referring to Table 1, the steps on which, in my opinion, tissue-culture might impinge are the following:

1a. Production from natural sources. These might include cells, animal and plant. Probably isolated normal cells would compare adversely with their source in rate and quantity of production, but mutants or hybrids might be discovered *in vitro* with economic possibilities (99) or substances differing slightly from the *in vivo* product may be produced (32).

1b. Identification or characterization tests for purity and stability. These are necessary for comparing repeated experiments. In some cases detailed and specific biochemical changes could be used.

2. Biological screening and acute toxicity. Tissue culture can give additional information regarding direct cellular effects, and control experiments can be carried out with already existing, related drugs. Obviously, however, animal tests are also necessary, and no manufacturer would attempt to have a product licensed without them.

TABLE I Stages in the Development of a Drug[a] (reproduced by permission of the World Health Organization from WHO Technical Report Series No. 563. 1975. p. 11).

1. Synthesis of new chemical or production of new material from a natural source	*Identification* of the new chemical or *characterization* of the new material Establishment of degree of purity and preliminary stability studies on the compound
2. Biological screening and acute toxicity	
3. Pharmacodynamic studies in animals	Development of the pharmaceutical formulations (dosage forms)
4. Pharmacokinetic studies Long-term toxicity studies started in animals	Initial stability studies on dosage forms Development of assay methods for active and inactive ingredients Development of initial specifications for ingredients
5. Early studies in man Completion of long-term toxicity studies in animals Special toxicological studies as required	Development of pharmaceutical formulations
6. Controlled therapeutic trials Continuation of special toxicological studies	Completion of stability studies and development of final specifications for the ingredients and formulation

[a] The difficulties involved in presenting this complex subject in the form of a small table are recognized. However, it was thought worth while attempting to summarize the activities required in the development of a new drug up to the point of registration. Each group of activities, both biological and pharmaceutical, shown at any step would usually be undertaken concurrently.

3a. Pharmacodynamic studies in animals. These are not replaceable by *in vitro* systems.

3b. Formulation. This is one area where there has been a surprising lack of tissue-culture testing. It is obvious that the other ingredients in a formulation will affect drug release and duration of effect. Such other ingredients are binding. disintegrating. and lubricating agents in tablets; tablet coatings; the various ointment bases; suspending. stabilizing. and emulsifying agents in suspensions and emulsions; preservatives in injections and ophthalmic preparations; and coloring and flavoring additives. Tablet disintegration tests alone are not adequate measures of drug availability at the required site. but different formulations can be observed in some cases for a direct effect on appropriate cells such as beating heart cells or ciliated mucosa cells. Many new pharmaceutical adjuvants are becoming available all the time. Some are quite sophisticated chemical substances. It is sensible to test them on their own, as drugs are tested, and also in complete formulations.

4a. Pharmacokinetic studies. The absorption, distribution, biotransformation, and excretion of drugs is not amenable to modeling in tissue-culture except in very limited ways. Cell uptake by phagocytosis or pinocytosis by various cell types may be compared, and stability in a tissue-culture system measured, but obviously pharmacokinetic studies are primarily an *in vivo* problem.

Long-term toxicity tests. As seen below, where examples of work with various drugs are cited, there have already been many studies on cellular toxicity, especially of anticancer substances.

4b. Development of assays. As in studies on identification, a particular biochemical effect may be used, in this case quantitatively.

6b. Stability tests. Cell systems can be used to monitor decomposition products for toxicity.

EXAMPLES OF DRUGS WHOSE EFFECTS ON CELLS HAVE BEEN STUDIED

The categories of drug on which the greatest amount of *in vitro* work has been carried out are antimicrobial and anticancer. In the case of the former, it is important to compare the toxicity of the drug toward the microorganisms and toward their "host." In the latter it is logical to compare effects on normal and malignant cells in searching for a drug selective for the latter. However, many other types of drug have also been investigated (47).

There has been much work on antimicrobial drugs, cell systems being used for simple toxicity tests, for more detailed mode of action studies, for studies on killing intracellular bacteria, and for lymphocyte transformation in allergy diagnosis. There are obvious advantages in using human material, as some species of microorganisms, or strains of species, affect only man. Also, in culture, large numbers of experiments can be set up to study mixtures of an antimicrobial substance with any one of many other substances, to investigate synergism or antagonism. It is a fact that many early antimicrobials were as toxic to epithelial cells and blood cells as to microorganisms, but this was not appreciated until Fleming's experiments on penicillin, which he found did not lessen white blood cell function (64). It is interesting that originally he was not looking for a drug, but for a tool to separate resistant and susceptible bacteria. This is an early example of an originally biological study having a therapeutic application.

Tissue culture can be used too to study factors modifying the effect of an antimicrobial substance, as in our own work with Bacitracin, in which we studied the effect of medium composition, presence or absence of various metallic ions, and storage in solution on the action on various cell types (50a and 52).

In the cases of chloramphenicol and tetracycline, retrospective tissue-culture studies, suggested by untoward findings in clinical use, indicated that the adverse effects might have been foreseen had the tissue-culture work been done first. In the case of chloramphenicol, marrow-culture work on inhibition of iron incorporation into haem might have indicated a danger of aplastic anemia (169). In the case of tetracycline, production of chromosome breaks might have indicated possible teratogenicity (176), fatty change in cultured hepatocytes might have indicated the same *in vivo* (189), and lessened protein and thymidine uptake in cultured bone might have indicated bone-growth derangements *in vivo* (15,106). These conclusions would not necessarily have been drawn from the tissue-culture work, but an alerting to their possibility would have been provided.

The acridines are a good illustration of the changing type of tissue-culture experiment within any one drug group. The early experiments measured toxicity by cell-killing or diminished phagocytosis, later work examined more detailed effects on chromosomes and nucleoli, and a recent paper (166) uses X-ray crystallography to visualize intercalation of a drug into DNA.

Anti-cancer substances have probably been the subject of more tissue-culture papers than any other group of drugs. Massive screening programs have been undertaken by the Cancer Chemotherapy National Service Center (CCNSC) Screening Laboratories. These originally were reported in *Cancer Research* Supplements and since 1967 have appeared in *Cancer Chemotherapy Reports*. The nature of the tissue-culture part of the screening test has evolved over the years, and it is a far cry from the state in 1961 when it was said that "the application of human cell cultures for the evaluation of cytotoxic activity of new carcinostatic agents has been reported by few investigators" (31). Before the first CCNSC test was set up, much preliminary work was required, comparing *in vitro* and *in vivo* results and evaluating the increased yield of active *in vivo* substances obtained if an *in vitro* prescreen were used. Comparisons of *in vivo* and *in vitro* results are still being carried out, of course, to achieve more valuable prescreening methods (170). Recent work by Thayer *et al.* (155) found that a mammalian cell test gave better correlation, both for active and inactive substances, with *in vivo* tests than was obtained between a microbial test and *in vivo* tests. The usual index measured is culture protein. Other systems measured cell numbers or change in morphology by a scale of numbers (160,161). A range of doses in geometric progression can easily be added to find the dose killing 50 percent of the cells or inhibiting protein production by 50 percent.

That the design of a tissue-culture test is by no means yet final was shown in the recent paper of Freshney and Paul (70), who emphasized the cell heterogeneity of many tumors and the effect of cell density on the result. The difficulty in designing a test was mentioned also by Dickson (55).

Much of the work on anticancer drugs is designed to find out the part of the cell cycle at which they act, so that useful combinations may be made, either with other drugs or with irradiation. Such tests came into existence following the discovery of ways to synchronize cells, which are fully discussed in Zeuthen (187) and Cameron and Padilla (24). Recent reports on the value of screening on synchronized cells was given by Fonken (66) and Bhuyan and Fraser (18).

Another way in which tissue culture may be used in relation to anticancer drugs is to find which of the available drugs is best for an individual patient's tumor by sensitivity testing, which is akin to antibiotic sensitivity testing. It may not be possible to predict from histological similarity alone that one tumor will be sensitive to a drug to which an apparently similar tumor was sensitive. In fast-growing cancers, such as melanoma, simultaneous screening against a range of drugs has obvious advantages. The test, of course, must be one giving a result rapidly. We have found the differential respirometer a useful apparatus for this purpose.

Antiinflammatory drugs have been widely studied too, as mentioned under the discussion of rheumatism. An account of this is given in Chapter 5 of Dawson's *Cellular Pharmacology* (47). More recent work relates the fall in prostaglandin production to the action of various antiinflammatory drugs (89,156,168). Vane and co-workers (168) performed their experiments in cell homogenates, on isolated cells (platelets), and in whole spleen perfused *in vitro*. The three systems all led to the same conclusion, that some of the therapeutic effects of various antiinflammatory drugs were related to inhibition of prostaglandin synthesis. The paper of Peters *et al.* (116) reports effects varying with the drugs used (phenylbutazone, indomethacin, sodium salicylate, and mefenamic acid) on cAMP levels and glycosaminoglycan secretion in fibroblasts both unstimulated and stimulated with PGE_1.

Anesthetic drugs at first seem unlikely candidates for testing by tissue-culture models, but again they are a group where retrospective tests have been carried out. The reports on hepatic dysfunction and increased toxicity with nutritional deficiency, associated with halothane, led to tests on cultured liver cells to elucidate or confirm the reports. A more realistic, albeit nonspecific, cell response investigated has been on beating cilia in mucosal cells.

Morphine tolerance was reported by Corssen and Skora (37), i.e., ability of cells to withstand doses lethal to cells that had never been in contact with it. They described also dependence, i.e., unhealthy cells on withdrawal. We have noticed very active pinocytosis on returning WI-38 cells from medium containing 0.075 mg/ml of morphine sulphate to medium without morphine. A more recent work by Sharma *et al.* (145) reported inhibition by morphine of adenylate cyclase activity, the morphine-sensitive cells having more narcotic receptors than resistant cells. They concluded that PGE-stimulated adenylate cyclase appeared to be the primary site of morphine action. They propounded a hypothesis for the molecular basis of narcotic addiction and tolerance, based on the morphine inhibition of adenylate cyclase leading to a fall in cAMP, with a subsequent compensatory shift restoring its level. The cell would then be morphine-dependent, as it would have an unduly high cAMP level on morphine withdrawal. This is a most elegant piece of experimental planning and deduction obtained from the detailed biochemical study of cultured cells.

Although cAMP is not a "drug" in the same sense as the other substances mentioned under pharmacology, it is relevant to include it here. There have been recent publications on cAMP too numerous to cite. Many are to be found in *Advances in Cyclic Nucleotide Research* (75). The editors refer to this substance's unifying large segments of the biological and medical sciences and pointing to more rational drug design in many fields. The relation of cAMP to cancer and psoriasis has already been mentioned. Other areas discussed by contributors to the above volume are immunity, epidermal proliferation in wound healing, cardiac contractility, and smooth muscle relaxation in asthma and bronchitis.

There has been a recent review on new drug possibilities based on cAMP (5). The possibilities are described systematically under the sections on drugs affecting adenine cyclase, which synthesizes cAMP; drugs affecting phosphodiesterases, which degrade it; analogues to mimic or antagonize it; and substances acting between its formation and functioning. Another example of a new drug based on cAMP was reported in the work of Cotton *et al.* (38) on a derivative more effective in some tumor systems than cAMP itself. The search was for improved cell penetration and metabolic stability.

Elegant organ-culture experiments were recently performed by Pratt and Martin (121) on the fusion of apposed edges of palate in rat embryos. They have added to knowledge of the biochemistry of this state in embryogenesis. It is interesting too to note the work in 1973 of Saxén (140) on the effects of hydrocortisone on the development *in vitro* of secondary palates in mice following reports of cleft palates caused by cortisone in mice and rabbits but not in rats and humans.

Another group of about 25 substances ubiquitous in tissue-culture work are the cytochalasins. These were originally isolated from molds in a search for anticancer drugs, but they do not have this effect. Their name means "cell-relaxing substances," and their greatest use is as a tool in cytological research. They were first examined on cells by Carter (26). They have a remarkable range of effects of cells. They inhibit cleavage, the cleavage furrow relaxing and the cell becoming first binuclear and then adding one nucleus for each "mitosis." They affect cell locomotion, the cell developing ruffles all around instead of having them only at the leading edge. Some concentrations cause nuclear extrusion, enabling survival of the remains to be studied for up to 3 days. Hexose transport through membranes is also inhibited. Thus they can be used in studying many events in the cell cycle of normal and abnormal cells.

Under pharmacology one must consider not only drugs and formulations of drugs, i.e., medicines, but also toxicity tests of surgical dressings, prosthetic materials, dental materials, and containers for pharmaceuticals. Recently it has been found necessary to test for toxicity plastic containers of injections and ophthalmic preparations. A report of Wilsnack *et al.* (181) compared cell culture and animal tests on rubbers and plastics, finding in most cases a high degree of correlation. They stressed the need to give full details of any cell system used and considered that the accumulation of further correlation information would be a worthwhile objective.

CONCLUSION

There is undoubtedly scope for much wider use of tissue-culture methods to add to our imperfect knowledge of many, diverse aspects of biomedical reseach. However, if tissue-culture tests were to be considered not as adjuncts to, but also as replacements for, some existing whole animal tests, then much more work requires to be done to establish just what is being measured in both types of test, the exactness of the measure, and the relevance of both indices to the proposed use of the material.

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S. FEDOROFF

In Vitro Systems in Medical Research

Tissue-culture deals with cells arranged in various degrees of complexity, removed from the organism and grown or maintained under controlled conditions *in vitro* (1). In the absence of a blood supply, the cells are nourished through diffusion of nutrients from the surrounding medium, and consequently the size of the explant is limited to a thickness of not more than 1.0–1.5 mm (2). It is possible to culture separated cells, small fragments of tissues, embryonic premordia of organs, and even whole embryos provided they are small enough for the nutrients to diffuse through them. Systemic influence is lacking in tissue cultures, and not all aspects of the behavior of cells in cultures correspond to those in the intact organism. Cells and tissues in cultures are isolated from the organisms and therefore are no longer subject to systemic influence. Tissue-culture experiments give information that cannot be obtained from animal experiments (3).They can tell us the direct action of an agent on cells or tissues, but, on the other hand, they can tell us nothing about systemic effects (3).

Tissue-culture as an isolated system in which cells live, reproduce, perform their function, age, and die is a science in itself, but as a technique it is applicable to research in many areas of biology. My viewpoint is not the science of tissue culture, but rather how various functional attributes of cells in culture have been utilized for investigations in the field of medicine.

CELL GROWTH

As cells grow in cultures, they divide mitotically and increase in cell number. These aspects of cell life in cultures have been utilized for specific studies.

Mitosis

Great interest in the study of human genetics was stimulated by a combination of three developments: the discovery of sex chromatin (4), the development of tissue-culture methods for the study of chromosomes (5), and the finding of an extra chromosome in patients with Down's syndrome (mongolism) (6,7). One of the most dramatic findings in studying dividing cells in cultures was the discovery by Tjio and Levan in 1956 that human cells have 46 rather than 48 chromosomes (8), as had been accepted for 33 years (9). This finding was possible because cells in cultures can be arrested in mitotic division and then can be prepared in such a way that the chromosomes spread out and can be individually examined (Figure 1).

It has been learned that at fertilization a large number of zygotes with chromosome abnormalities are formed. Depending on which chromosome or chromosomes are involved, developmental anomalies occur in varying degrees of severity. In some cases the zygotes are lost even before they implant into the uterus; in others the implantation takes place but the embryological development can proceed only up to a certain point, after which the embryo, or foetus, depending on the stage of development, dies and is aborted. In still other cases, the development proceeds, and individuals are born with multiple abnormalities. Again depending on the chromosomes involved, the life span of such chromosomally abnormal individuals may vary: some die within a few days or weeks after birth, some in early adulthood, and some may live on with their abnormalities (10). Nowadays, all these abnormalities can be detected by culturing *in vitro* either skin biopsies or cells obtained from aspirated amniotic fluid during the early stages of pregnancy. Since abnormalities can be detected even before birth, the parents can be counseled about what can be expected and whether or not therapeutic abortion is indicated.

Using tissue-culture methods, it is now possible to demonstrate banding patterns on chromosomes of most cells of the body (Figure 1A). The patterns are specific for each individual chromosome and serve as landmarks within it (11,12). These advances not only allow the counting of the numbers of chromosomes per cell, but also the detec-

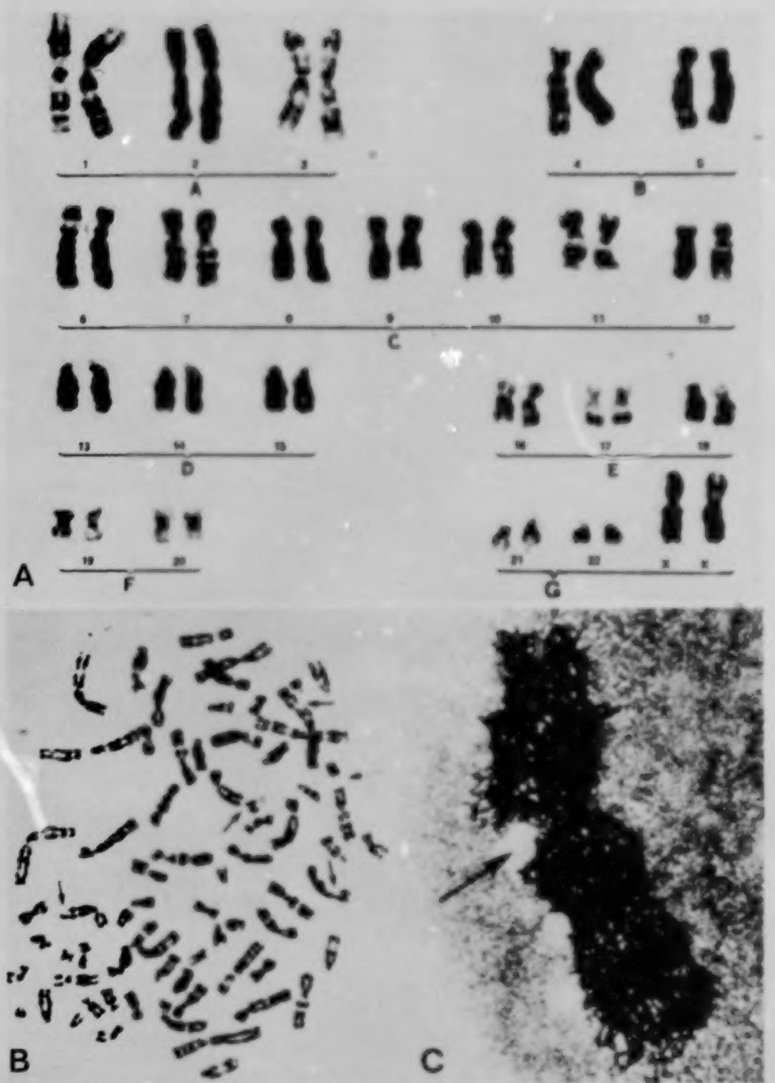
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FIGURE 1 (A) Human female karyotype. Each chromosome can be identified by its gross morphology and banding patterns. Courtesy, Dr. H. C. Wang. (B) Human chromosomes of one cell of a human cell line (HeLa) showing chromatid aberrations (arrows) due to radiation in cultures. Courtesy, Dr. H. C. Wang. (C) An electronmicrograph of one human chromosome showing the banding pattern and radiation damage (arrow). X15,618. Courtesy, Dr. G. D. Burkholder.

tion of intrachromosome rearrangements and even the observation and determination of the precise site of the breakage of chromatids due to radiation (Figures 1B and 1C) or certain chemicals (13,14). This means that by taking a sample of blood or a small skin biopsy, growing the cells in cultures, and analyzing their chromosomes, minute chromosomal aberrations can be detected.

At present, there is a coordinated international effort to use tissue-culture methods for scoring chromosome abnormalities in order to quantitatively monitor human exposure to ionizing radiation and other environmental mutagenic factors (15).

Increase in cell number

The most dramatic event of cell (not organ) cultures is that the cells may double in number within a few days. If one would continue growing the cells without any elimination, in no time they would overgrow the laboratory. The fact that cell cultures can be frozen and stored at low temperatures (− 198°F) and that at any time the cells can be thawed out and grown again in cultures allows the study of cells even after a patient from whom the small number of cells originally were isolated is no longer available. (There are several cell banks that supply frozen cells of various types on request anywhere in the world.)

It is also possible to grow cells in large fermenters for industrial purposes, such as the propagation of viruses for vaccine production or for the synthesis of economically important compounds. For example, human embryonic kidney cells can be grown in large cultures, and, by appropriate manipulation of the medium, they can be stimulated to an increased production of urokinase, an enzyme used to activate the lysis of blood clots. Urokinase produced in tissue culture is being tried now clinically (L. J. Lewis, personal communications).

In addition to mammalian cells, plant cells are of great importance for the industrial production of medically useful substances. For example, plant cell cultures have been used to produce the antitumor alkaloid camptothecin, which was found to be effective on mouse tumors as well as human tumor cells grown in cultures. The genus *Camptotheca* includes a single species of tree, *Camptotheca acuminate*, from the People's Republic of China, which contains only a small amount of the alkaloid. The calus of this tree was grown in suspension cultures as single cells, and such cultures produce the alkaloid, which has been isolated and purified (16).

Similarly, a large number of other compounds have been produced, among them various enzymes, antiviral agents, antibiotics, glycosides, sterols, and pharmacologically important agents such as atropine, scopolamine, nicotine, ergot, and others (17,18). In addition to the synthesizing of compounds by plant cells, such cultures can be used for biotransformation. Plant cell cultures have been used to biotransform progesterone, pregnenolone, testosterone, digitoxin, and other compounds of medical importance (18). The potential of plant tissue culture unfortunately still has not been fully realized. I have no doubt that in the future plant tissue culture will have a most profound effect on human life.

PHENOTYPIC EXPRESSION

The recent finding that skin fibroblasts in cultures in many instances retain the biochemical defects observed in patients opened a whole new avenue of investigation for diseases due to inborn errors of metabolism. These diseases are characterized by a lack of functional enzymes, thus causing either absence of an end product of a metabolic pathway, a pile-up of substrate in the pathway proximal to the block, the presence of excessive amounts of metabolites, or secondary effects of the metabolic distortions on regulatory mechanisms in the same or other pathways (19). There is a long list, continually growing, of genetic errors in carbohydrate, amino acid, and lipid metabolism and other metabolic pathways that are being studied with the help of tissue culture (20). Such studies have led to the diagnosis of diseases before clinical symptoms appear, in some cases to the development of therapy and in others to clarification of the disease process, and consequently to diagnosis and therapy. I shall give some examples.

Tay-Sachs disease is an autosomal recessive disease, i.e., both parents are usually normal. The affected children develop symptoms 9–12 months after birth and die between the ages of 3 and 5 years. In Tay-Sachs disease, there is an accumulation in the neurons of a lipid, ganglioside Gm_2, resulting in a gradual degeneration of the nervous system. By the age of 1 year, the affected children already have some physical and mental deterioration (21). The disease was found to be due to the lack of an enzyme, hexosaminidase A (Hex A). The absence of the enzyme was first demonstrated in blood serum and leukocytes and also in skin fibroblasts growing in tissue culture (21). Subsequently, Okada and O'Brien demonstrated that Hex A is found in normal cultured fibroblasts and cell cultures from amniotic fluid, but is absent in the cells from affected children and present in lowered amounts in cells cultured from parents of affected children (21). These observations provided a means for prenatal diagnosis of the disease. Now there is a fluorimetric assay available by which the enzyme can be detected in a drop of blood or cells. The method is automated, and mass screening for the heterozygous carriers is being done (21). In this case tissue culture played an important part in the identification of the deficient enzyme.

Cystinosis is an autosomal recessive trait in which there is excessive storage of cystine in cellular organelles (lysosomes) and deposits of cystine crystals in the kidneys and eyes of patients. In some forms of the disease there is growth retardation, rickets, and renal failure. Three phenotypes of the disease have been described: a fatal infantile form (Type I), a benign adult form (Type II), and an adolescent form (Type III) of intermediate clinical severity. Clinical prognosis may be correlated with the degree of cystine storage (22). In cultures, skin fibroblasts from such patients retain the ability to accumulate cystine. It was found that Cleland's reagent dithiothreitol (DTT) can penetrate cells in cultures and diminish intracellular free cystine intracellularly without affecting other amino acids or the viability of the cells (22,23). Following experiments with tissue cultures, this drug was given intravenously to a terminal patient who tolerated it well, and the cystine concentration in the body cells decreased (22). An oral preparation given to two patients with the infantile form of the disease reduced the cystine concentration in their peripheral blood leukocytes and stabilized renal filtration function. When DTT was stopped, there was a steady reaccumulation of cystine in leukocytes, and renal function began to deteriorate (24). In this case tissue culture was used to select and test the effect of a therapeutic agent on cells.

The mucopolysaccharidoses are a group of diseases in which mucopolysaccharides accumulate in the lysosomes of various body cells. These diseases are due to autosomal recessive genes that must be acquired from each parent, except for one type that is sex linked (Hunter's syndrome). Although they all have certain clinical features in common, they can be distinguished by clinical and biochemical findings. The clinical features of mental and or physical retardation may not appear at birth or in early infancy, and the life span varies from one type to another, as well as with the severity of the disease. Seven types of the diseases have been described and defined by clinical and biochemical findings. When skin fibroblasts from patients with Hurler's or with Hunter's syndromes, and in some cases from heterozygous individuals, are grown in tissue cultures and stained with toluidine blue dye, purple granules appear in the cytoplasm of the cells (metachromasia). No such granules appear in comparable normal cells. Subsequently, it was found that when cells from two patients of clinically different types of mucopolysaccharidoses were grown in cultures together, there was intracellular reduction of accumulated mucopolysaccharides. In other words, each cell type corrected the defect of the other. When cells of all seven types of diseases were cultured with each other in different permutations, it was found that some corrected the defects of other cell types and others did not. The explanation was that in this group of diseases deficiencies of different enzymes are involved and that each type of disease is due to the defect in or lack of one of these enzymes. Cells that could not correct each other must have had the defect in the same type of enzymes.

As a result, these seven diseases have been reclassified. The Sanfilippo syndrome was found to be two distinct diseases, because cells from some Sanfilippo patients cross-correct cells from other Sanfilippo patients. It was also found that Hurler's syndrome and Scheie's syndrome were due to a defect in the same enzyme and therefore were two forms of a single disease (19). The defect in the cells from patients with mucopolysaccharidoses could also be cross-corrected when grown with normal cells, and the corrective factor (enzyme) from normal, as well as cells from patients, was present in the culture medium. In other words, the defect of the one type of cells could be corrected by the addition of medium in which either normal cells or cells of a patient with a different type of the disease were grown (25,26). Five of these factors have been isolated, purified, and indeed identified as specific enzymes; one has been tentatively identified; and one needs further work. Each of the enzymes is specific for cells of a patient with a specific type of the disease (26).

How can this finding be applied? The problem is to get a sufficient amount of enzyme for administration, prevent its degradation before it reaches the defective cells, and ensure that cells *in vivo* utilize it. Patients were transfused with leukocytes from a normal individual in the hope that the transfused cells would release the missing enzyme, which could be taken up by the cells of the patient. In another trial, the cell-free plasma from normal individuals was transfused. I understand that the results so far are encouraging but not definitive (25).

The story of the mucopolysaccharidoses demonstrates the effectiveness of tissue culture in the investigation of human disease at the cellular level. As a result, we can now diagnose the mucopolysaccharidoses prenatally and take steps to prevent the development of abnormal individuals. In the future the disease will almost surely come under complete control.

In the three examples discussed so far, the enzyme deficiency has been identified, and the therapy is based on providing the cells either with the missing enzyme or a substitute. A different approach has been used with the cells of patients with galactosemia. An attempt was made to introduce the gene that could code for the missing enzyme by infecting cells with a selected virus. The application of this principle of genetic engineering has been successful in cultures, but so far not in patients (27).

TRANSFORMATION—TUMORIGENESIS

Immediately after explantation, tissues and cells in culture begin to adapt to the new environment. Upon release from the control of the organism, and depending on the degree of disruption of histotypic architecture of the tissue, the cells begin to divide, increase in numbers, and at the same time gradually lose many of the specialized functions performed *in situ*. Eventually the cells die or become transformed. Transformation is more common to cells of some species than others, e.g., cells of murine origin transform readily but those of man do not (28). On transformation, the cell morphology changes, the growth rate increases, cell-to-cell relationships are modified, and in some instances, but not all, the cells produce tumors when injected into compatible hosts (28). Tumor cell formation in cultures seems to be part of a process of cell transformation that may take place even in a chemically defined medium where no known inductive agents are present (29). Cells in culture may acquire the property of tumorigenicity and with time may also lose it partially or completely (30,31). It seems that changes within the cell can occur in response to the milieu in cultures as part of an attempt to acquire autonomy, i.e., to free themselves from restraints imposed by homotypic (similar) or heterotypic (dissimilar) neighboring cells. The process of tumor-cell formation can be considerably speeded up by infecting cultures with viruses, treating cultures with a carcinogen, or by irradiation (28). During the process of cell transformation [a rather confusing term (1,29)], many changes in the cultures occur; however, which changes are part of adaptation to culture conditions leading to acquisition of the ability to grow indefinitely *in vitro* and which are related to acquisition of tumorigenic properties is difficult to sort out (29). Transformation is a gradual process, and acquisition of tumorigenic properties is not necessarily the terminal stage of such a process (32).

One of the key questions is how the process of tumor-cell formation (neoplastic transformation) in cultures compares to transformation of normal cells to tumor cells *in situ*. In cultures, neoplastic transformation takes place as part of the cellular response to disorganization of histological architecture of the tissue and its response to the culture milieu (32). *In situ*, neoplastic transformation takes place within a histological structural arrangement and environmental conditions compatible with the normal structure and function of neighboring cells. When cells from human or animal tumors are explanted in cultures, they adapt to the tissue-culture milieu without the necessity of losing the differentiated state they had *in situ*. One of the most dramatic examples of this is that human thyroid carcinoma cells, which secrete thyrocalcitonin, when explanted in tissue culture, continued secreting even after subsequent additional transformation by SV-40 (33). Tumor cells in culture seem not only to retain the differentiation they had before explantation, but may even undergo further differentiation. For example, neuroblastoma cells in tissue culture may acquire differentiated neuronal properties. This has been demonstrated on morphological, physiological, and biochemical grounds (34). Tumor cells explanted into tissue culture do not seem to respond to the tissue-culture environment in the same fashion as normal cells, which with time lose their differentiation. It seems that, although the end result of neoplastic transformation *in vitro* and *in situ* may be the same, the process leading to the acquisition of tumorigenicity is not (32).

Since malignancy is an invasive process of cells with destruction of normal tissue in the immunologically competent host, the only test for neoplastic transformation in tissue culture is the injection of cells into a compatible host.

Because invasiveness and destruction of normal tissue are criteria of malignancy, considerable work has been done in tissue culture using a three-dimensional matrix populated with embryonic or normal cells in order to study the interaction between tumors and normal cells *in vitro*, but there still is some uncertainty as to whether such interactions *in vitro* are equivalent to those *in situ*.

The present state of the study of tumors in tissue culture can be described by Robert Frost's words: "We dance round in a ring and suppose, but the secret sits in the middle and knows." We see tumor cells forming, but we don't know how this happens; we know a great deal about the behavior of tumor cells, but we don't know what is related to tumorigenic properties and what to other properties of the cells. In spite of this, by careful study of cells in cultures, it should be possible in some cases to give a prognosis for the donor patient. However, this is far from routine laboratory procedure and can only be done satisfactorily by a few experienced people.

There is no doubt that tissue culture has been a very effective technique in cancer research and that its use in probing tumorigenesis will eventually lead to a number of diagnostic and prognostic procedures.

AGING—A FINITE CELL LIFE SPAN

Cells in cultures proliferate and increase in number, but this can't go on forever unless the cells undergo transformation. Not every animal species has cells that can transform spontaneously (28). Hayflick and Moorehead noted that diploid cell cultures of the human fetal lung, regardless of the culture milieu, can survive in continuous cell propagation for only 40–60 population doublings (35,36). The actual lifetime of a culture depends on the age of the tissue donor. It has been shown that cells from the skin of young individuals can survive in cultures longer than those from older individuals (37), and it has been proposed that senescence of cells in tissue culture is related to aging in man and animals and that the life span of proliferating cells is a programed intracellular event under genetic control (38). Recently, a method has been devised by which the proliferative capacity of the cells in cultures can be estimated, and it has been speculated that such a method eventually may be used to estimate a relationship between the biological and chronological age of humans (39).

Disorders such as Werner's syndrome, progeria, diabetes, and Rothmund's and Down's syndromes are associated with premature aging, and skin cell cultures from such patients either do not live in cultures as long as cultures from normal individuals (37,39–42) or have a decreased percentage of cells capable of forming small cell colonies in tissue culture (43).

Although the above-mentioned observations are intriguing and show a relationship between the aging process in the whole organism and behavior of cells in cultures, the problem of aging seems to be much more complex, i.e., cells from different tissues do not have the same life span in tissue cultures (35), and there may be no relationship between the life span of the animals of a species and the proliferative capacity of their cells in cultures. For example, cells from animals of a species with a maximum age of 3 years may undergo 170 doublings in cultures, whereas cells from animals of a species with a maximum age of 46 years may double 82 times in culture (44). As previously mentioned, man's cells double approximately 40–60 times (35,38).

In any given culture there is a considerable degree of heterogeneity in the proliferative capacity of cells (46). It seems that the life span of cells in cultures can be modified. Hydrocortisone added to human embryonic fibroblasts extends the life span of cells in cultures (45). Use of tissue cultures in the study of the relationship between senescence of individual cells to that of the whole organism is most intriguing and full of promise.

DIFFERENTIATION

There are two major technological approaches to tissue culture. One of them, cell culture, is to dissociate tissues into single cells and grow them in cultures as a cell population without an overall histological organization. The other approach, organ culture, is to maintain the histological relationship between the cells by explanting fragments of tissues into cultures. All cultures can maintain a certain degree of differentiation, particularly if isolated from adult tissue or tumors (32,47); however, the tendency is toward dedifferentiation, degeneration, or transformation. In organ cultures tissue architecture is preserved, and, although the cells may divide, they do so within an organized framework. In such cultures cells can maintain their function and differentiated state much more readily than in cell cultures. If undifferentiated tissues from an embryo are grown in organ culture, they may even continue differentiation *in vitro*.

Embryonic tissues

Tissue cultures have been extensively used in the study of embryonic processes by following the differentiation of organ premordia (48), or even whole embryos (49), under various culture conditions.

For example, mouse zygotes as young as the two- or four-cell stage can be explanted in culture, and these cells will develop *in vitro* into embryos having a cardiovascular system with a beating heart, nervous system, and other organ systems. Such embryos can develop in cultures only up to a point; then degeneration of cells begins, and the embryo dies (49,50).

In our laboratory we were able to isolate precursor cells of the nervous system (neuroepithelium) from very early stages of chick embryos (Figures 2A and 2B). Such undifferentiated cells could differentiate in culture into neurons (51,52) (Figure 2C). There are many examples of similar observations. A dramatic example of the application of tissue culture to developmental processes is the study of the development of the brain in neurological mutants of the mouse. For example, the reeler mutant (rl) is an autosomal recessive mutant in which mice have instability of posture and gait. The mice have grossly

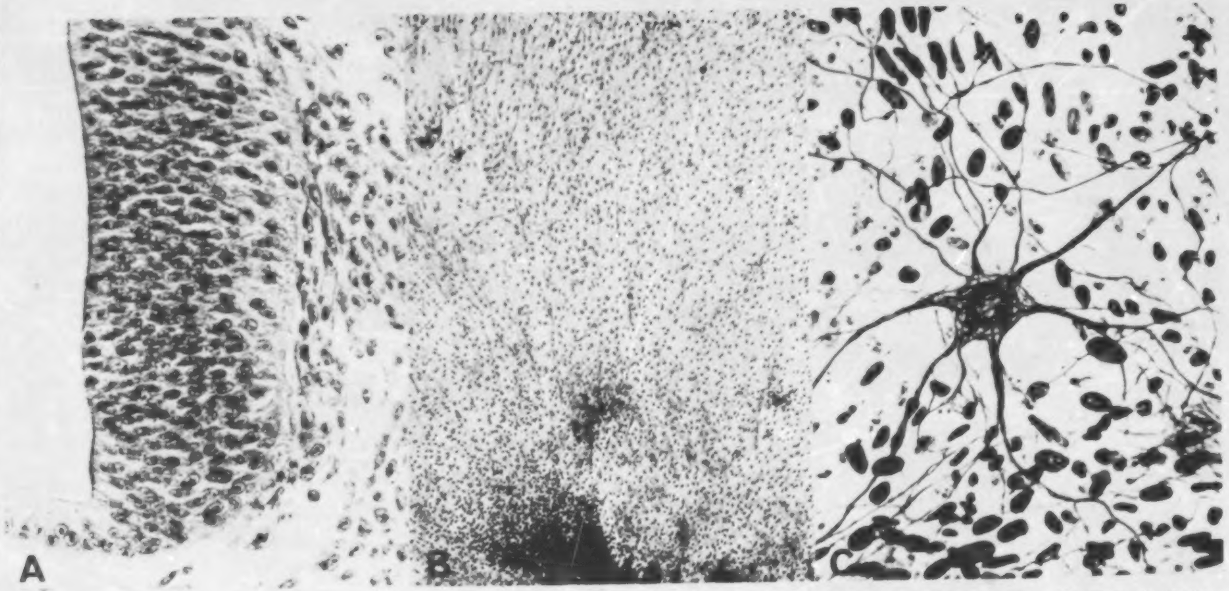

FIGURE 2 (A) Section of chick embryo (stage 19) neural tube (spinal cord) hematoxylin and eosin stain X500. Courtesy, Dr. K. R. S. Fisher. (B) Monolayer of cells grown from an explant of neural tube (stage 17) after 21 days in culture. Bodian silver stain X60. Courtesy, Dr. K. R. S. Fisher. (C) Differentiated neuron in culture of neural tube cells after 21 days of culture. Bodian silver stain X600. Courtesy, Dr. K. R. S. Fisher.

abnormal brains and commonly die about 3 or 4 weeks after birth (53). It is possible to dissociate cells from the brains of such mice at late embryonic stages. In cultures, dissociated cells can be made to aggregate to form small spheres within which the cells sort themselves out and reestablish a pattern of cellular organization. Dissociated cells of mutant mouse brain in such preparations reestablish the pattern seen within the brain of mutant mice, indicating that the genetic defect is mediated directly by the neurons of that part of the brain (54). Such experimental models allow a study of developmental processes that, on the one hand, may explain how malformations take place, and, on the other, may provide an insight into developmental processes of the normal brain.

Differentiated cells

Differentiated cells are much more difficult to grow in cultures. However, in order to investigate specific aspects of the function or pathology of organs or organ systems, it is necessary to use differentiated cells. In many instances differentiated cells have been grown in cultures, and much valuable information has been obtained. For example, there is great interest in the pancreas because of its involvement in diabetes mellitus, a disease in which there is a dificiency or a disturbance in the action of insulin produced by the so called "beta cells" in the islets of Langerhans. Methods have been developed to isolate islets from the pancreatic tissue of various animals (55,56). In tissue culture the beta cells have been grown primarily as cell cultures, in which they secrete insulin and respond to glucose concentration in the medium (57,58). Although glucose, tolbutamide, glucocorticoids, growth hormone, and glucagon all apparently stimulate beta-cell replication *in situ*, only glucose and tolbutamide stimulated replication *in vitro*. One explanation is that the first two act directly on beta cells and the other three agents act *in situ* on other tissues, which in turn affect the beta-cell replication (59).

Another approach has been to grow beta cells in a perfusion system. A device has been manufactured that consists of a large number of small hollow fibers (Figure 3A). Cells can be grown on top of the three-dimensional matrix of these fibers (Figure 3C) and the nutrients perfused through the hollow fibers, which act as capillaries (Figure 3B). The walls of the fibers are permeable to a molecular size up to 100,000 mol wt. In such systems a very large number of beta cells (beta cells from 180 newborn rat pancreases per culture) have been grown, and they have secreted insulin and responded to glucose concentration

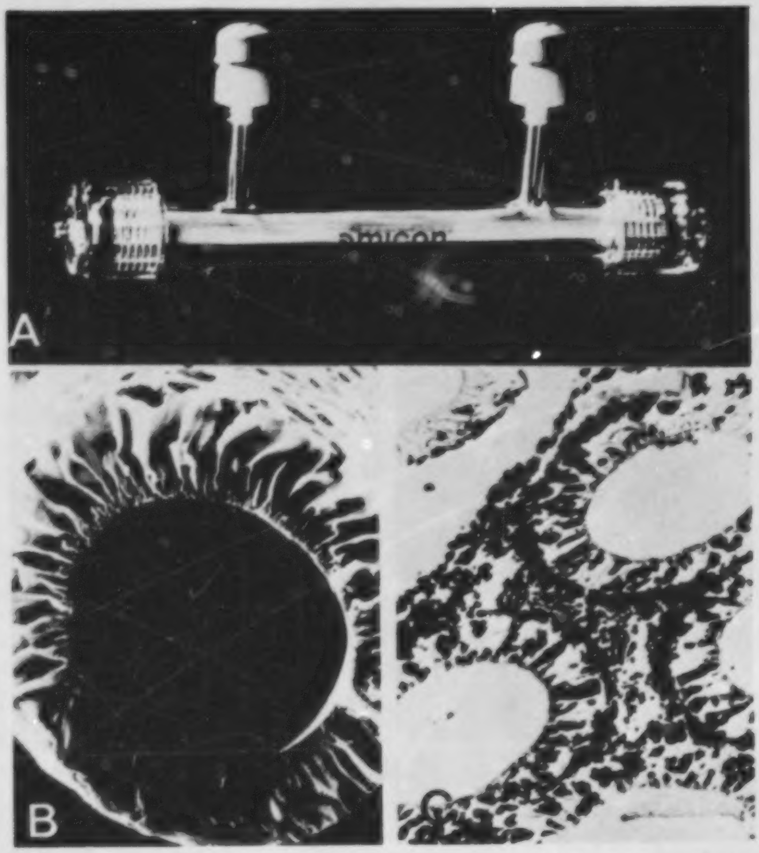

FIGURE 3 (A) A device for tissue culture. Horizontal tube contains a bundle of artificial capillaries that form a three-dimensional vascular system permitting better nutrition of the culture. Courtesy, Amicon Co. (B) Cross-section through one of the artificial capillaries showing spongelike walls through which nutrients diffuse. Courtesy, Amicon Co. (C) Culture of rat hematoma cells. Cells grow between the artificial capillaries. Courtesy, Dr. C. Wolf.

in the medium (58). This may be a prototype for a future artificial pancreas.

Recently it has been possible to isolate islets of Langerhans from human postmortem pancreatic specimens and grow them intact in cultures (Figures 4A–4D). Such organoids contain alpha, beta, delta,

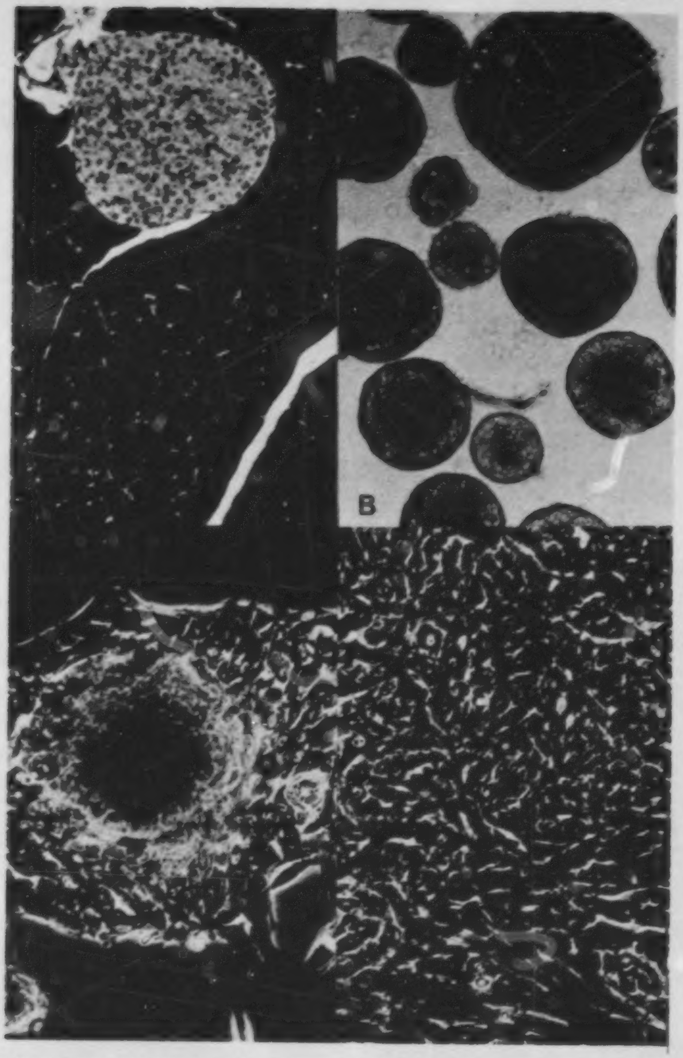

FIGURE 4 (A) Section of pancreas showing islet of Langerhans. (B) Isolated islets of Langerhans from human foetal pancreas, grown in culture for 14 days. Courtesy, Dr. H. Goldman. (C) Human fetal islet of Langerhans developing a monolayer of endocrine cells. Courtesy, Dr. H. Goldman. (D) Monolayer of endocrine cells originating from an islet of Langerhans. Courtesy, Dr. H. Goldman.

and ductal epithelial cells, all normally found in islets of Langerhans *in situ*, and secrete insulin, glucagon, and agents that resemble gastrin and somatostatin. They have been maintained in cultures for as long as 3 months (60–62). Such cultures will be most useful to study hormone production and control mechanism.

Many attempts have been made to transplant islets of Langerhans to animals having experimental diabetes. The liver seems to be the best site (63,64). The isolation of islets of Langerhans from cadavers and growth in cultures for appreciable periods of time makes it possible to collect amounts large enough for transplantation. Although the whole problem of transplantation of islets of Langerhans is immense, no doubt tissue culture will play a major role in its solution.

An example of a somewhat different use of differentiated cells is the application of tissue culture to the investigation of atherosclerosis.

Blood vessels consist of endothelium, smooth muscle cells, and fibroblasts. It is possible to prepare pure cultures of all three cell types, although the first two are at present of major interest.

Endothelial cells in cultures can be identified by a number of criteria and can be maintained in cultures for up to 5 months (65,66,67). Recently it has been shown that human endothelial cells in cultures secrete one of the antihemophilic factors, the von Willebrand factor ($\mathrm{VIII}_{\mathrm{VWF}}$). This factor, obtained from medium in which endothelial cells were grown, can correct dificiencies in platelets obtained from patients with von Willebrand's disease (68,69). This finding supports the importance of the platelet–endothelial cell interactions and provides means to further explore these interactions in normal and pathogenic situations (69).

Smooth muscle cells from the walls of aortas or large arteries can be grown in cultures (65,70). They produce myofilaments *in situ* and *in vitro*, deposit elastic and collagenous fibers between the cells, and secrete mucopolysaccharides (65,71). Smooth muscle cells are implicated as the main proliferative cells in the formation of atherosclerotic plaques (72,73). Therefore, a considerable amount of work is in progress to study the response of smooth muscle cells to various fractions of blood serum lipoproteins from normal individuals and from patients with various types of hyperlipemias. Detailed biochemical work on cholesterol synthesis in smooth muscle and endothelial cells in cultures is now possible, as well as studies of the regulation of cholesterol synthesis by various lipoproteins in serum, the penetration of lipoproteins into the cells, and their subsequent fate (74–76). Cells from human atherosclerotic plaques have been grown in tissue culture (77), and it should be possible to study the stepwise changes of smooth muscle cells into the so-called foam cells, which form atherosclerotic plaques.

The proliferative potential (finite life span) of smooth muscle cells from the arterial wall, uterus, wall of the duodenum, atherosclerotic plaques, and tumors of the uterine wall (leimyoma) are being studied (77,78), and provocative hypotheses have been proposed. They should stimulate an inquiry into the possibility that smooth muscle cells in different parts of the vascular system may be at different stages of their life span and therefore may have different susceptibilities to injury and atherosclerosis, or that there are similarities between atherosclerotic plaques and smooth muscle tumors.

In connection with the studies of blood vessels, the attempt to develop cardiovascular prostheses that could effectively replace diseased parts of blood vessels should be mentioned. Tubes resembling blood vessels are manufactured from nonporous polyurethane lined by microfabric made from a variety of materials and providing a three-dimensional matrix for cell attachment (Figures 5A and 5B). Cells from various sources are grown in cultures and then planted into the microfabric of the tubes. Eventually cells may form multiple layers of a very smooth lining (Figure 5C). Such cell-lined prostheses are tested in specially devised equipment under conditions simulating a circulatory system (79,80). The ultimate goal is to be able to develop cardiovascular prostheses lined with autologous cells, i.e., the patient's own cells.

HYBRIDIZATION

One of the most interesting observations made in tissue culture is that two cells may fuse into one, their chromosomes mix, and form one nucleus. Such cells can proliferate and form a progeny of cells (81). This observation was the beginning of the development of one of the most powerful tools in the study of genetics.

It was found that cells could be fused under controlled conditions to produce cell hybrids between different cell types and even between cells of two far-removed animal species (82). In such hybrids chromosomes of both species are functional and are expressed phenotypically. For example, a hybrid between human and mouse cells will express both human and mouse phenotypes. In these studies, as a bonus, it turned out that in human–mouse cell hybrids the human chromosomes were so rapidly eliminated that it was possible to select clones of hybrids that retain only a few human chromosomes (83). By analyzing the segregation patterns of human phenotypes and specific chromosomes in hybrid clones of independent origin, it is possible to determine which phenotypes segregate together. It is logical to assume that genes that control the expression of two phenotypes that always segregate to-

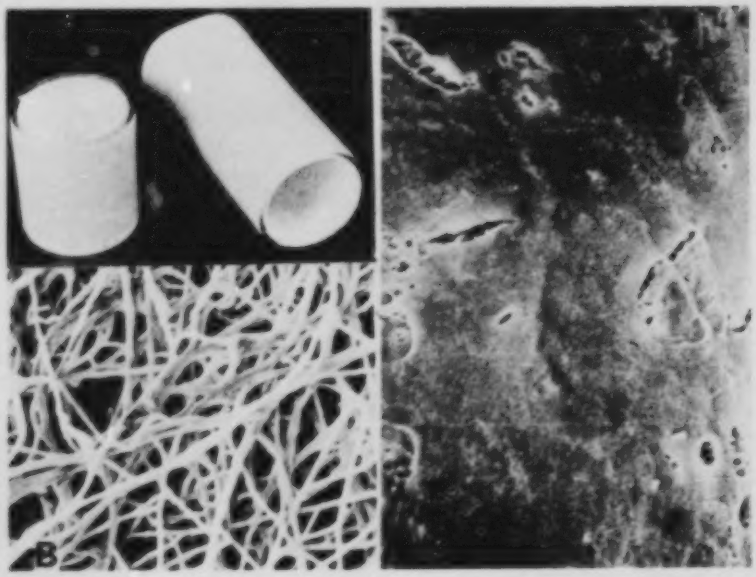

FIGURE 5 (A) Nonporous polyurethane tubes (18 mm in diameter) coated with nonwoven microfabric. Courtesy, Drs. R. H. Kahn and E. Burkel. (B) Scanning electron micrograph of microfabric lining polyurethane tubes. Each fiber is approximately 1 micron in diameter. Courtesy, Drs. R. H. Kahn and E. Burkel. (C) Scanning electron micrograph of human epidermal cells grown on microfabric lining of polyurethane tubes. Surface is completely covered and is very smooth. Courtesy, Drs. R. H. Kahn and E. Burkel.

gether must be closely linked on the same chromosome. If one correlates the segregation patterns of individual chromosomes and the particular phenotypes, then it is possible to assign a locus responsible for that phenotype to a specific chromosome. One can go even further. By studying cells with chromosomal translocations or deletions, it is possible even to determine the approximate position of the genetic locus on the chromosome (84). Recently, a new method has been proposed. Human cells are subjected to ionizing radiation before hybridizing them with rodent cells. Radiation breaks chromosomes, and the degree of breakage can be controlled by the dosage. By this method the segregation of phenotypes can be correlated to smaller fragments of chromosomes, thus determining more precisely how close two genes are to each other (85). The number of gene loci assigned to specific chromosomes and the regional chromosome mapping is advancing very rapidly. At the International Workshop on Human Gene Mapping, held in Rotterdam in 1974, it was reported that 108 loci have been assigned to specific autosomal chromosomes and that of these 43 have been located on specific regions on the chromosomes (86,87). No doubt by now the number has greatly increased.

The cell hybrids have been used not only for gene mapping of human chromosomes, but also to study the genetic basis of malignancy (88), the mechanism of gene expression in cell differentiation (89), rejuvenation of aged cells (90), production of cells that could secrete antibody of predefined specificity (91), and the genetics of human disease (92). Now evidence is also accumulating that cell hybridization also occurs *in vivo* between tumor and normal cells (93,94). The application of this technique in the future is limited only by imagination.

SUMMARY

This paper has reviewed the use of tissue-culture methods in medical research and considered their use and limitations in the future. It contained some examples to demonstrate the role tissue-culture has played in the acquisition of present-day knowledge in such areas as chromosome diseases, inborn errors of metabolism, cancer, aging, neurological disorders, diabetes, and atherosclerosis.

The main limitation of tissue-culture is that cells or tissue are grown in an artificial environment outside systemic control. However, since the whole purpose of tissue-culture is to make possible the study of cells and tissues in isolation, this disadvantage is also its advantage. Superficially, it would seem that the milieu in tissue cultures is controllable, but in reality there are so many factors involved that at present it is difficult to control them all for any length of time.

In the future, tissue-culture will become even more scientific, and refinements in tissue-culture methodology, together with other methods, will greatly increase the precision and resolution of the study of the cell. The application of tissue-culture to biomedical research will be limited only by the ingenuity and inquisitiveness of those in the field.

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JOHN C. PETRICCIANI, HOPE E. HOPPS,
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Application of *In Vitro* Systems to Public Health

This paper will consider the specific application of *in vitro* systems to protect the public health from the perspective of a federal regulatory agency that has the responsibility to assure that viral vaccines and other biological products are safe, effective, potent, and pure. Even within this limited context, the use of *in vitro* systems is in many respects inseparable from a broader consideration of the use of animals and animal cells in both basic and clinical research. It is because of developments in this field that *in vitro* systems have become important in the production and quality-control testing of viral vaccines.

Since 1798, when Edward Jenner first demonstrated that cowpox would protect against smallpox, animals have played an important role in the development of vaccines. They have been used for the production of vaccines and as models for human diseases. It was the serial propagation of the rabies virus in the central nervous system of rabbits by Louis Pasteur in 1884 that led to the production of his inactivated vaccine against that disease. To this date, vaccinia virus, propagated on the scarified skin of the calf, sheep, or rabbit, and rabies virus, usually grown in the central nervous system of rabbits, are still important sources of vaccines used in various parts of the world for protection against smallpox and rabies, respectively. The development of other vaccines paralleled closely the advances made in the cultivation of animal cells *in vitro*. It was only after Max Theiler was successful in propagating the yellow fever virus in minced chick and mouse embryo cultures and after it was realized that the fertile hen's egg was a naturally packaged cell-culture system that the 17-D vaccine against yellow fever was obtained. Perhaps the greatest stimulus to the use of cell-culture systems extensively for the study of human and animal viruses was the discovery by Enders and his co-workers that poliovirus could be propagated in tissue cultures of nonneural origin (1). This finding led to the development of vaccines against paralytic poliomyelitis and set the stage for the use of other cell-culture systems in vaccine research and production.

Even though it is convenient to focus attention on different areas of research, such as basic, clinical, and applied, for the purposes of justifying commitment of resources, we all know that they can not be defined in a mutually exclusive fashion and that each of them is to some degree dependent on the others for new insights and directions. The story of the poliovirus vaccines just mentioned can be used to illustrate this point. Figure 1 presents in a simple diagrammatic form the interrelationships that can occur among basic, clinical, and applied research and provides three specific examples that show that the flow of events

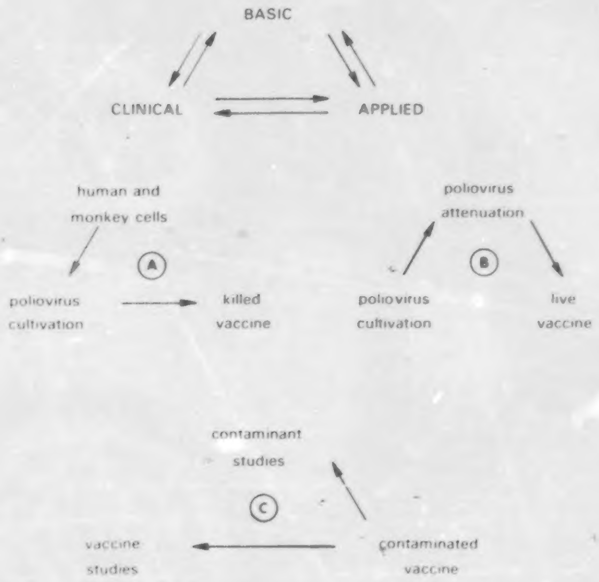
BASIC
CLINICAL
APPLIED
human and monkey cells
A
poliovirus cultivation
killed vaccine
poliovirus attenuation
B
poliovirus cultivation
live vaccine
contaminant studies
C
vaccine studies
contaminated vaccine

FIGURE 1 Examples of interrelationships among basic, clinical, and applied research.

can go in essentially any direction. In example A, the basic research effort of establishing nonneural human and monkey cells in culture allowed the clinical effort of cultivating the three different types of poliovirus *in vitro* to proceed, and that in turn led to the introduction of the Salk, or killed, poliomyelitis vaccine. In example B, the clinical finding of *in vitro* poliovirus cultivation led to basic research efforts to attenuate the virus, and that success was soon translated into the Sabin, or live, poliomyelitis vaccine, widely used today. And finally, in example C, applied research has demonstrated the presence of extraneous agents in the vaccines.

In the late 1950's, SV-40 was shown to be present in the killed vaccine, and more recently bacteriophages were found in the living vaccine. Each of these discoveries led to concerted basic research efforts to study the contaminants themselves in order to assess whether or not their presence in the vaccines constituted a risk to the public health. Clinical research studies of the involved vaccines were also carried out to define the magnitude of the problem and the circumstances that might be associated with the introduction of the contaminants in order to eliminate them in the future. These examples simply serve to illustrate the point that the flow of research information is frequently multidirectional, with events at the basic, clinical, or applied levels often having an impact on each of the others.

The remainder of this paper will deal with selected practical applications of *in vitro* systems that relate directly to public health and will attempt to demonstrate that society realizes clear and concrete benefits from *in vitro* systems that have been developed from basic and clinical research efforts.

Virus vaccines are familiar consumer products to most of us in the United States, usually because of personal experience with them. In the United States, the control of viral infectious diseases such as poliomyelitis and rubella has been a major public health accomplishment that would have been impossible, or at least delayed by many years, without the availability of *in vitro* systems. In this area, animal cells have played a major role, not only in the production process, but also in the regulation and control of the vaccines themselves.

Table 1 shows the live viral vaccines now licensed for use in humans in the United States, the year of licensure, and the laboratory hosts used for each. One might ask why cells from such a variety of species are used to produce the vaccines. Two of the most important determinants in the initial selection of a specific cell system for vaccine production are the susceptibility of the cells to the virus in question and the yield of virus following its replication in the cells. There are

TABLE 1 Human Virus Vaccines Currently Licensed in the United States

Vaccine	Year	Laboratory Host
Smallpox	1798	Man–bovines
Rabies	1885	Rabbit
Yellow fever	1935	Mouse Chick embryo
Typhus	1938	Chick embryo
Flu	1940	Chick embryo
Mumps (inact.)	1945	Chick embryo
Poliovirus (inact.)	1955	Monkey kidney cells
Poliovirus (live)	1961	Monkey kidney cells
Measles (live)	1963	Chick embryo cells
Mumps (live)	1967	Chick embryo cells
Rubella (live)	1969	Duck embryo cells Rabbit kidney cells
Poliovirus (live)	1971	WI-38 cells

obviously many additional considerations that are necessary before a vaccine can be regarded as acceptable for human use, but the two basic points of susceptibility and yield are the *sine qua non* factors in the development of a vaccine and are the principal reasons for the variety of cells now used in vaccine production.

Primary cultures of monkey kidney cells, that is, those derived directly from monkey kidney tissues, were the first *in vitro* systems in which a licensed poliovirus vaccine was prepared; because these cells were found to be very susceptible to the virus, and the yield of each of the three types of poliovirus made vaccine production a practical undertaking. Table 2 illustrates this point. The usual yields of the three types of poliovirus are high enough to give from 40 to 300 human doses from each milliliter of the original virus harvest. Poliovirus vaccine is

TABLE 2 Relationship of Poliovirus Yields to Vaccine Dose

	$TCID_{50}$ per ml		
Polio Type	Harvest	Vaccine Unit Dose	Trivalent Vaccine Doses per ml of Harvest (Approximate)
1	$10^{7.6}$	$10^{5.9}$	40
2	$10^{7.5}$	$10^{5.0}$	300
3	$10^{7.6}$	$10^{5.7}$	80

also a good example to illustrate the basic steps involved in the use of cell cultures for the production of biologicals. This is shown schematically in Figure 2. In this scheme, each of the monkeys is tested for evidence of tuberculosis and quarantined for a period of at least 6 weeks. After the animals are sacrificed, they are examined for any abnormalities, particularly for signs of tuberculosis and herpesvirus simiae (B virus). The kidneys are removed to establish cell cultures that will eventually be inoculated with an attenuated vaccine strain of poliovirus. Following the multiplication of the virus in the monkey kidney cells, the fluids containing the virus are harvested and tested for safety, purity, and potency. These procedures are carried out both at the vaccine manufacturer's laboratories and at the Bureau of Biologics, Food and Drug Administration, before the vaccine is released for medical use (2). The tests for safety, purity, and potency all include the inoculation of a variety of cell cultures for the detection of extraneous agents, as well as for biological markers characteristic of the attenuated vaccine strains. Even though this discussion deals principally with *in vitro* systems, it is important to point out here that the single most

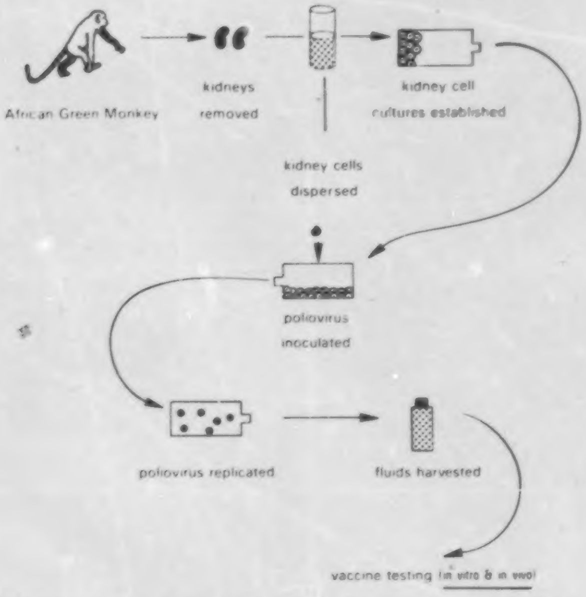
African Green Monkey
kidneys removed
kidney cells dispersed
kidney cell cultures established
poliovirus inoculated
poliovirus replicated
fluids harvested
vaccine testing (in vitro & in vivo)

FIGURE 2 Basic steps in the production of live poliovirus vaccine from monkey kidney cells.

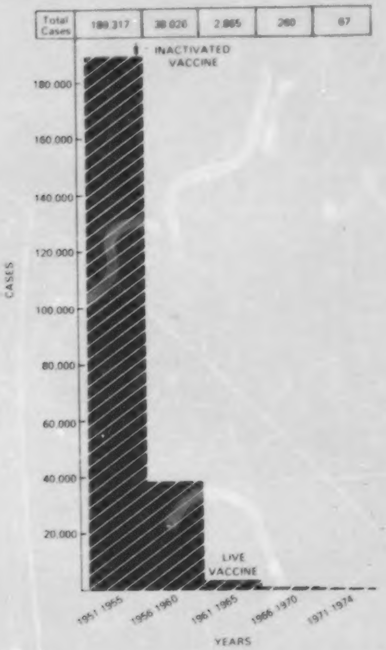

FIGURE 3 Incidence of paralytic poliomyelitis in the United States during the period 1951 through 1974.

important safety test for the oral poliovirus vaccine is an *in vivo* test in monkeys. This is done to assess the neurotropic properties of the vaccine in order to ensure that the virus has not increased in virulence during production of the vaccine.

Since 1962, it has been required that each lot of live poliovirus vaccine be inoculated into the brains of 30, and spinal cords of 15, *Macaca* monkeys and that after a 17–21-day observation period the brains and spinal cords be examined histopathologically for evidence of viral neurovirulence. In order for a given lot to pass the test, it must be demonstrated to be no more neurovirulent than a reference virus preparation tested in the same manner.

The impact that both the inactivated and live vaccines have had on the incidence of poliomyelitis in the United States is shown in Figure 3. The dramatic drop in the number of cases of polio from about 190,000 between 1951 and 1955 to only 67 between 1971 and 1974 depended on the availability of both animals and animal cells to produce and test the vaccines (3). If poliovirus vaccine is to remain available to protect the public health, then we must continue to rely on the use of both animal cells and animals themselves in production and testing, because there are no satisfactory substitutes at the present time.

In addition to the use of cells in vaccine production, there are two other public health areas that should be mentioned briefly in which cell cultures play an important part. *In vitro* systems have been used recently in the screening of drugs, food additives, and a variety of other substances for their potential to cause mutations and cancerlike changes. Interest in this application is widespread, and considerable time, money, and effort have been devoted to its exploration by the National Cancer Institute, the Environmental Protection Agency, the National Science Foundation, and the National Institute of Environmental Health Sciences, as well as the Food and Drug Administration. Satisfactory screening systems should be rapid, reproducible, inexpensive, and reliable complements to more complex *in vivo* studies. Cell-culture systems offer at least the potential for meeting each of those criteria when applied to mutagenicity and carcinogenicity testing. The types of chromosomal analyses that are now possible and that were discussed by Dr. Hsu are excellent examples of how findings in basic research can be directly applied to mutagenicity screening. *In vitro* test systems are still in the developmental stages, but they seem to be emerging as important adjuncts to the more familiar animal tests and will probably assume an increasingly prominent role in protecting the public from unnecessary exposure to mutagens and carcinogens. However, it is much too premature to place total reliance on *in vitro* tests. *In vivo* animal tests will continue to be required to fully assess the safety of various agents for man.

Another example that should be cited in a discussion of the applications of *in vitro* systems to public health is their use in the diagnosis of viral diseases. The single most important contribution to the field of virology was the development of cell-culture systems for the isolation and characterization of viruses. When Enders, Weller, and Robbins discovered that the three types of poliomyelitis could be grown in cells of nonneural origin, a whole new horizon opened for virologists. Before that time, isolation of viruses was done by inoculating specimens into laboratory hosts such as adult and suckling mice, guinea pigs, and embryonated eggs. There are a number of viral agents that can only be detected in cell cultures because no animal systems for their isolation have been found. On the other hand, viruses such as influenza are best isolated in embryonated hens' eggs, while cell cultures are poor alternates. Table 3 lists some of the viruses of public-health importance and

TABLE 3 *In Vitro* Isolations of Selected Viruses of Public Health Importance from Clinical Specimens

	Cell System	
Virus	Highly Sensitive	Alternate
Polio types 1,2,3	Primary monkey kidney	BS-C-1
Measles	Primary human embryonic kidney	HEp-2
Rubella	Primary monkey kidney	Primary rabbit kidney
Influenza	Chick embryo	Primary monkey kidney

representative cell-culture systems used to isolate those viruses from clinical specimens (4). In many cases, primary cell cultures prepared from human embryonic or monkey kidneys are the most sensitive assay systems. There are problems with both of these primary cell systems. The monkey is sacrificed to obtain the kidney tissue, and the use of human embryonic tissue is embroiled in social, moral, and ethical issues concerning the embryo, the fetus, and abortion. However, some established cell lines of humans, such as HEp-2, and African green monkeys, such as BS-C-1, are almost as sensitive as the primary cultures.

If we accept the premise that animal cells, and in some instances animals themselves, are necessary to produce and test products such as vaccines that have a direct impact on the public health, then one might ask whether or not there are alternatives to the use of primary cell cultures that would conserve animals. One alternative was developed in 1961 by Drs. Leonard Hayflick and Paul S. Moorhead. They showed that human embryonic lung cells could be established *in vitro* and propagated through about 50 serial subdivisions while maintaining a normal chromosomal constitution over the first two-thirds of their finite life span (5). In addition, one of these diploid cell lines, WI-38, was shown to be susceptible to a number of human viruses. Since that time, WI-38 cells have been used in viral isolation studies and diagnostic work throughout the world, and in 1971 live poliovirus vaccine produced in WI-38 cells was approved for use in the United States. One of the special advantages of the WI-38 cell system is that an enormous number of cells can be derived from a small primary pool if that pool of cells is allowed to go through serial subdivisions and is then stored in the frozen state until needed. This procedure is illustrated in Figure 4 and with some modifications is the one that has been used to provide a continuous supply of cells that were originally derived from one embryo 15 years ago.

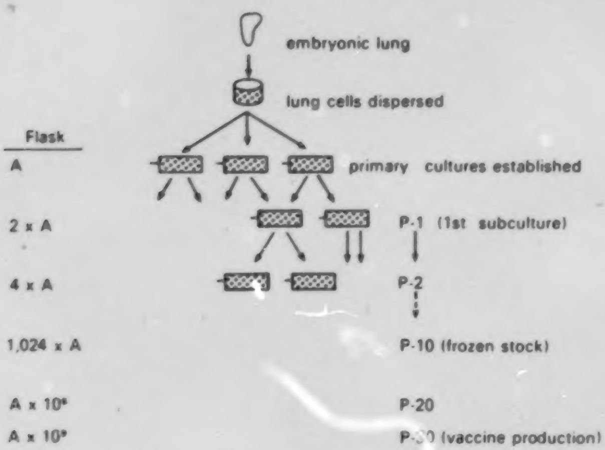

FIGURE 4 Basic steps in the development and use of diploid cell systems.

The cells from the tissue are first dispersed and then seeded into a number of flasks to establish what is termed the primary cultures. Each of these primary cultures can then be divided into two (or more) daughter cultures designated passage 1 (P-1). This process can be repeated to give a geometric progression with a potential of about 1,000 flasks from each primary flask by the tenth passage. At that time the cells can be pooled and frozen in many small vials in liquid nitrogen. The vials of frozen cell stock can in turn be thawed as needed and the cells reestablished in culture for further propagation. The process can then be repeated to form a secondary frozen pool at passage 20. The cells remain useful for vaccine production through about passage 30. At that point an enormous number of cells is potentially available for use because of the conservation of the very early passages.

While WI-38 has been extremely useful in a wide variety of programs, ranging from aging studies and cell biology to vaccine production, it is nevertheless limited in total production potential and in its viral susceptibility. Because of these constraints, and in response to a recommendation of the International Conference on Cell Cultures for Virus Vaccine Production in 1967, we initiated a program to develop alternatives to the WI-38 cell line (6). Special emphasis was placed on the development of cell lines from rhesus and African green monkeys and rabbits, three species that have been useful in vaccine production.

We recognized that an undertaking such as this was going to be a long-term effort which by its very nature was exploratory and was without guarantees of success. Experience has shown that it is, in fact, possible to develop from a rhesus embryonic lung a diploid cell line very comparable to WI-38, and the results of this work have been reported (7). Although the program has not yet been completed, the results thus far suggest that it may also be possible to develop African green monkey and rabbit diploid cell lines. Each of these cell lines has been shown to be susceptible to a number of human viruses, and at least some of them may find a useful place in biologics production or control.

The conversion from the use of primary cell cultures to the use of cell lines wherever possible would result in the conservation of many animals, since the production of primary cultures almost always requires the death of the animal donating the tissue. There does not seem to be a strong current trend in that direction, however. For example, a recent letter by a cell-culture supplier suggested that, because of the diminishing availability of rhesus monkeys, their customers should consider switching from primary rhesus cells to primary cells of two other monkey species. Obviously, such a switch would still result in the sacrifice of monkeys—only the species would be different. There was no hint of a suggestion that monkey or human cell lines should be tried as substitutes for the primary cells. There may, of course, be justifiable reasons in certain cases for the use of primary cells rather than a cell line. Those special instances, however, need to be distinguished from all others, and primary cells should not continue to be used simply because of tradition, convenience, or habit. What is needed is an effort to educate both the suppliers and users of cells of their responsibilities to explore reasonable alternatives to the use of primary cells.

However, if this is to be accomplished, a variety of well-characterized normal cell lines should be available for use in biomedical research and development, because no single cell system will be applicable to all of the potential needs. As mentioned earlier, we have supported efforts to establish cells from two nonhuman primate species and one nonprimate species; but continuing efforts to develop additional human and animal cell lines are certainly in order and should be supported by a variety of federal agencies.

Finally, some mention should be made of the future of *in vitro* animal systems as related to public health. The past history of the interrelationships among basic, clinical, and applied research illustrated earlier in this talk, together with the much larger general experience with cell cultures in the biomedical sciences, points out the unpredictability of the sequence of events that can eventually lead to significant improvements in the public health. Having said that, however, let me venture to speculate about the future by posing a few questions rather than trying to make predictions about where benefits might be derived from *in vitro* animal systems.

Biologists have now been able to culture a variety of cells with specific functions such as the pancreatic islet cells, which produce insulin. Such insulin-producing cell cultures from mice have been transplanted successfully into diabetic mice with apparently good control of the diabetic state (8). Do experiments such as this suggest that in the future we will be able to treat human deficiency diseases not by replacing the biochemical that is lacking but by transplanting normal cells capable of producing the missing biochemical *in vivo* under normal physiological control mechanisms?

The use of *in vitro* systems in genetics, and especially gene therapy, has attracted more and more attention since the original studies describing the *in vitro* correction of a human genetic defect by using a virus to transfer normal genes to the mutant cells that lacked those genes (9). Do such *in vitro* gene-therapy experiments mean that we are on the verge of correcting at least some of the genetic diseases of man for which we have had available only partial therapy or none at all?

The recent identification of a virus in human leukemia cells (10) opens the possibility that a causal relationship exists between the virus and the disease in man similar to that already known for several animal systems. Will it be shown that such a human leukemia virus can be grown not in human cells but in nonhuman primate cells such as those that we have developed, thus paving the way for the first virus vaccine directed against a human malignancy?

It is, of course, impossible to do other than speculate on those potential applications of *in vitro* systems; but they do serve to illustrate the point that animal cells are finding increasingly broad use in the biomedical sciences and that we can expect public-health benefits to eventually emerge from at least some of them.

Animal cells have been and will continue to be extremely useful in the diagnosis and prevention of a number of infectious diseases. Their continued availability and use are likely to bring additional benefits to society in the treatment of certain metabolic and genetic diseases and possibly cancer. In certain cases, however, animals themselves continue to be the only choice for testing the safety of biological products, and their availability has an immediate and direct effect on the public health.

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DISCUSSION

STEVENS: I would like to ask Dr. Petricciani about quality control of marketed pharmaceutical drugs. There are requirements, as I understand it, by the Food and Drug Administration that LD_{50} values be repeated on each batch of drugs produced. Is it necessary to continue doing this, which causes suffering we are most concerned with and also wastes animal life? Is it valid to require an LD_{50} test, rather than accepting a chemical specification drawn from methods that modern technology offers in those cases where it is a good chemical method?

PETRICCIANI: The LD_{50} test *is not* required for quality control on each batch of drugs produced and marketed in the United States. To the best of my knowledge, it is required for only three out of thousands of licensed drugs on a batch-by-batch basis. Those three are antitumor drugs in which the antitumor activity is closely correlated with the toxicity so that the LD_{50} becomes a very real measure of its safety in man.

KURTZ: I had a question of Dr. Dawson. It refers to the promising and ambitious uses of tissue-culture in drug development and safety testing, which I think must be tempered with some of the actualities of our ignorance in pharmacology as it stands in the traditional sense. In practice, many pharmacologically active substances are detected by rather indirect animal models. For example, in a model designed to detect drugs with antianxiety activity, there is a milk-drinking procedure. Fortunately, this is not a lethal procedure for the rat, but the rats don't normally drink milk when stressed or anxious. When given drugs with putative antianxiety activity, increased milk-drinking is one of the features that occurs in a pharmacological screen. We don't really test for specific behavioral activity in the rat with drugs that turn out to have specific behavioral modification in the human. We find by serendipity, or pure accident, that certain kinds of drugs will, in fact, produce these effects.

If we resort to the simple expedient of comparing a potential new candidate —let's say an antidepressant drug—with a highly specific standard as exemplified in your split image, we run the risk of producing a "me-too" drug. If we put it against a standard such as imipramine, which is a standard acceptable antidepressant drug, and get a positive correlation with it, the chances are you are going to get another drug with qualities like imipramine. We are not necessarily interested in finding another imipramine with its weaknesses, but rather we are interested in something new with superior qualities.

In the split-imagery technique, it seems to me there is another control that is desired. Should we not have the addition of a positive control, necessary to give us some impression of the nonspecificity of the effect that we see to temper our preconceptions of a positive effect, if you will? Could you comment on that, Dr. Dawson?

DAWSON: This is one of the fundamental problems in any biological method of assay, whether it is whole-animal method or cellular method. The response used in practice in the assay is not necessarily the response that is being produced clinically. It is valid only to use a different response where it has been previously shown that the two are correlated. This is something you often have to do, for instance use a lethal test of the cardiac glycosides instead of a test-measuring force of contraction. It applies just as much to cell culture as to animals that you are often measuring something other than the effect that you actually want.

So far as cell cultures are concerned, if you were working with a drug that had a direct cellular effect you could measure it well, but an antidepressant is something you can't directly measure in cells at all.

KURTZ: That is right, so we must have in many cases what you would call a nonspecific effect. The cells have a certain limitation on their abilities to respond to most anything that alters their milieu in an unfavorable way. They have a limitation on the number of things they can do, so I would think that, as in any good experiment, the whole experiment is only as good as the controls built into it.

KRAMER: I had a concern and a question when I first learned about this symposium and was asked to participate in it. I buried it, but fortunately Dr. Hsu and Dr. Petricciani both mentioned items related to it. That encouraged me to bring it up again, because I think it is quite pertinent. It was mentioned by Dr. Hsu that the use of primate kidneys led to his concern about the killing of rhesus primates for the obtaining of kidneys for such studies.

He mentioned, and this was only a suspicion of mine, that this use grew to such numbers that there was concern about the rhesus population with regard to such studies. My question is directed, and I would like some answer, as to whether other tissue-culture studies and research have led to concern about animals. I would also like for Mrs. Stevens or somebody from the Animal Welfare Institute to tell me something about their position with regard to tissue-cultures. If they are promoted, what is their position with regard to the killing of large numbers of animals for this use?

KURTZ: May I make just one comment? There is always the possibility of working with one kidney from a rhesus monkey, if you are going to do cell lines from it, and leaving the animal alive.

HSU: Dr. Petricciani can answer this question much better than I can, but I would just like to mention a point. The kidney epithelial cells do not usually develop into cell lines; therefore, one must use the primary, or at best early secondary, cultures for either assay or production. If one can develop techniques to produce long-term kidney epithelium cell lines, then it would be perfectly reasonable to use them, because we would not have to kill many monkeys.

PETRICCIANI: The decreased availability of the rhesus monkeys is not primarily due to the use of the monkeys to establish cell cultures from kidneys, although that is certainly one aspect of their use. There are multiple other uses in the biomedical sciences for rhesus monkeys, and I think that the impact of all of these other uses is much greater than the use of monkey kidneys to establish cell cultures.

To my knowledge, there are no other species that are in short supply as a direct result of use of those animals to establish cell cultures. Someone else might correct me, but to my knowledge there are not.

KURTZ: I think there was a time when we were producing the polio vaccine in many different places. I hate to tell you how many rhesus monkeys were destroyed at Parke-Davis just for their kidneys to produce the Salk vaccine originally. We are not in that activity anymore, but I am sure there was, at one time, a great deal of pressure along this line.

DAWSON: May I say that to the best of my information, the monkey system has been replaced by the WI-38 and other lines for production now. I have heard it said cynically that it was only the frequency of occurrence of SV-40's that saved complete extinction happening to this particular species. As far as I know, there is no other species similarly endangered. My opinion is that, in cell material for testing drugs for human use, it is logical to use human cells.

KRAMER: Would Mrs. Stevens care to answer?

STEVENS: I can speak for our organization as being extremely interested in the protection of endangered species. There is an international convention that will sharply reduce commerce in endangered species. The necessary 10 countries have ratified it, so it is beginning to go into effect.

Again, I would emphasize that our primary concern with respect to research, testing, and production is prevention of pain, distress, and fear for all animal subjects. However, when endangered species are exploited for any purpose, we become extremely concerned. I think that you have heard from the panelists that tissue-culture techniques have never so far come near endangering a species.

In fact, the rhesus is not endangered, even though it has been used so very widely for so many different types of research.

T. A. LOOMIS

A Review of the Validity of Presently Accepted Scientific Standards

As I look at the various types of biological research involving whole-animal studies, two items become evident. One of these is that most people who are doing research involving whole-animal studies would very much like to have a simpler, much less complicated experimental system from which they could obtain the same or at least comparable data. Unfortunately the less complicated experimental systems such as *in vitro* systems, although acceptable for certain objectives, frequently do not supply the complete answer, and the researcher is left with the necessity of using whole animals as experimental subjects. The second factor that becomes evident as I review the nature of biological research involving whole animals is that each of these studies fall into one of three basic types. One type is directed toward a better understanding of a normal biological mechanism. These are the studies in the biochemical or physiological fields. A second type involves a better understanding of a disease process. These are the studies directed toward evaluation of nutritional, infectious, degenerative, genetic, and neoplastic processes. The third type involves a better understanding of the effects of exogenous chemical agents on the biologic systems. These are the studies that have long been conducted by pharmacologists and toxicologists on chemicals that have considerable economic interest, because some of them, such as drugs and food additives, are intentionally administered to humans, and other agents, such as agricultural chemicals or environmental pollutants, are potentially capable of reaching and affecting the health of humans. It is in the area of pharmacologic and toxicologic studies that I have been rather extensively involved for the past number of years. It is also in this same area that many of the so-called "standards" or "standard biologic tests" were developed and are currently used. Because of my experience in conducting some of these types of tests, this presentation will be concerned primarily with pharmacologic and toxicologic types of biologic tests.

The objective in studying the effects of drugs and other chemicals in expendable species of animals is based on the assumption that the data obtained can be extrapolated to unexpendable species such as the human being. Under the condition that certain basic principles of the subject of chemical–biologic interactions are recognized, this assumption is a valid one, and whole-animal experiments on expendable species has in the past and continues currently to be the principal experimental tool by which both the benefits as well as the health hazards of chemicals on mankind can be rationally evaluated. Therefore, this presentation will be concerned with some whole-animal biologic tests as they are conducted for the purpose of evaluating chemicals in so far as they may be related to beneficial or detrimental effects in humans. I will simply list most of them, but will go somewhat into detail regarding two.

In actual practice, the extent and type of experimental data that are desirable as far as any specific chemical agent is concerned depends on the ultimate intended use of the compounds. New drugs that may be consumed by humans on only one or two occasions may not need as extensive a study as would drugs that may be used on many occasions over a period of a number of years in the treatment of chronic illness. Food additives, food-coloring agents, or even pesticides, which may exist as residues in food, would necessarily be extensively studied on animals, in contrast to the minimal studies that may be done on a chemical that may be used only for industrial purposes. In the case of the new agricultural chemicals, experience in recent years has indicated that we need to study these agents for effects on many species before an evaluation can be made regarding the overall lack of toxicity to not only man but also to a wide variety of wild species that may eventually come in contact with the chemical agent.

When a new chemical agent is developed, it may be exposed to one or more of a large number of tests that are conducted on whole animals. In general, there are at least two major categories of whole-animal tests in pharmacology and toxicology. These are the types of tests that are designed to determine (1) the beneficial, i.e., pharmacological, as compared to (2) the harmful, i.e., toxicological, effects of chemical agents. The tests for beneficial effects are those that are applied to such agents as new drugs, in which case the nature of the drug action would determine which specific test might actually be involved for any given chemical agent. There are a large variety of such tests, some of which are very general and others of which are rather specific. Table 1 summarizes the nature of these tests. Among those listed in the tables, most consist of both animal and clinical tests, but in each case any new compound would of necessity be considered as a candidate for clinical testing only if it is already shown to be effective in the corresponding animal test. All of these tests might well be called tests that are standards by which the pharmacologist operates within his profession. In addition, the pharmacologist must acquire all of the information possible regarding harmful or undesirable effects, as well as information on absorption, distribution, and excretion by animals of the chemical agents that he studies. Although the pharmacologist acquires data on the specific actions (therapeutic) of drugs on animals, he also obtains information on the toxicity of the drugs. On the other hand, the investigation of drugs, as well as all other chemical agents, for potential to produce harm in animals is more systematically conducted by the toxicologist. There are both general and specific tests that are identified with toxicology.

The use of animals for quantitative determination of the toxicologic potential of chemical agents dates back to the very early 1800's, when Mathieu J. B. Orfila studied the harmful effects of chemicals and plant materials that were used in those days in the treatment of disease. The modern toxicologic tests have gradually developed since that time, but it was not until the pure food and drug laws became effective that systematic investigation of the biological effects of chemicals other than drugs received serious attention.

Thus, over the years certain types of toxicologic tests have been designed, modified, and improved until they have become accepted by

TABLE 1 Pharmacologic Tests (Animal and Clinical)

Antihypertensive
Vasodilator
Autonomic
Antiarrhythmic
Coronary vasodilator
Antiallergic and antianaphylactic
Analgesic
Anesthetic
Anorexic
Sedative
Psychomotor stimulants and depressants
Laxative
Blood coagulation
Blood cell formation
Diuretic
Antacid
Neuromuscular blockage
Antianxiety
Antidepressant
Anticonvulsant
Antiparkinson
Antiemetic

most toxicologists. This series of general types of tests can be conveniently divided into three major categories, dependent primarily on the duration of the test. These categories are the acute test, the prolonged test, and the chronic test. In general, the acute test involves administration of the chemical on only one occasion, although in a rare instance administration may be on two or three closely spaced occasions. It is by this test that the acute toxic and the lethal effects of a chemical are determined. The prolonged test always involves administration of the chemical on multiple occasions, usually on a daily basis for a period of not more than 90 days in the rat or mouse, but it may be for as long as a year in the dog. In the third test, the "chronic toxicity test," the chemical is administered, usually on a daily basis, for the major part of the duration of the life of the experimental animals. In the mouse or rat this would be 2 years and in the dog approximately 7 years.

In addition to the three categories of tests just listed, there are a number of specific tests that are usually conducted on compounds to which considerable human exposure is anticipated. This category of special tests includes tests for (1) potentiation with other chemicals, (2) effects on reproduction, (3) teratogenicity, (4) carcinogenicity, (5) mutagenicity, and (6) effects on the skin and eye.

A rather large number of tests are generally available for use in animals in order to evaluate the type of biologic effects that a compound may possess. They are designed to demonstrate pharmacologic or toxicologic effects and they are frequently conducted for this purpose; but it should be clear that the tests are also conducted to determine the order of safety of a chemical agent, because safety is simply the reciprocal of harmfulness.

None of the foregoing tests are conducted on very many compounds, as such extensive testing rapidly becomes economically impossible. However, for every compound it is desirable to have some information about its safety or harmfulness. The single test that is most feasible and is most commonly conducted for this purpose is the general acute-toxicity test.

The acute-toxicity test is conducted for estimation of all effects of the chemical including lethality, and it involves administering, on a single occasion, the compound to a sufficient number of test animals to enable determination of the average quantity necessary to produce death in the average test animal. The figure that is obtained is commonly called the LD_{50}, which means the *lethal dose* for 50 percent of the animals. Actually there is a great deal more information obtainable from this test than that associated with lethality. A brief description of the actual procedure that is generally followed when the LD_{50} is obtained will demonstrate the real meaning of the term and will demonstrate how other information is acquired by the test. It will also demonstrate why in the near future we will continue to need this test as one of the very basic standards in toxicology.

The acute-toxicity test used to determine the LD_{50} consists of administering the compound to the animals on one occasion. Its purpose is to determine the symptomology associated with administration of the compound and the order of lethality of the compound. Initially a species of animal is selected and a route of administration of the chemical is decided upon. As far as the selection of species is concerned, it would be ideal to base the selection on the principle that the species selected would possess anatomical, physiological, and biochemical systems similar to those of the human. Also, the selected species must be sufficiently economical and available so that the entire test is feasible. Most such tests are conducted initially on mice, not just because they are readily available, but also because they are relatively inexpensive to purchase and maintain and because they possess many of the anatomical, physiological, and biochemical systems found in man. Rarely are species other than mice or rats used for the determination of the LD_{50}.

Next the route of administration is selected, and this is ideally the route of eventual intended use of the compound in humans. However, even if the intended use of the compound does not involve the oral route, the oral route is used, if only for comparative purposes with other related compounds. Very probably the mouse will be used as the experimental animal, and it will be given the chemical by the oral route. If the chemical agent that is to be tested is a new agent about which there is no available biologic-effect data, it is necessary to conduct an initial rough dose-finding experiment. This initial experiment involves selecting doses (i.e., amounts) of the chemical, based on suspected toxicity of the compound, and administering them to a single or at the most two animals. By using logarithmic spacing of the doses in different groups of animals, a dose will be found that will be lethal to the animals. When this dose is found, an additional series of tests is conducted on the same species using at least four animals in each test group. The range of doses necessary to produce death is narrowed down to the point whereby death is produced in some, but not all, members of the high-dose group and symptoms of intoxication appear in some members of the low-dose group.

The proper conduct of an acute-toxicity test involves recording the occurrence of all sublethal effects as well as the "no apparent effect" dose of the chemical. Also, the animals are followed for at least 2 weeks after receiving the test chemical, so that, if delayed effects occur during this time, they can be observed and recorded. If these conditions are achieved, then the data are plotted and analyzed statistically for estimation of the LD_{50}. In this manner it is possible to rather precisely determine the quantity of the chemical agent necessary to produce death in 50 percent of the animals tested. Since the LD_{50} has been determined for many compounds, it is a rather basic index of the relative order of activity of chemical agents. One must be cautious about using these data outside of the context of the actual experiment. For example, if the route of administration of the compound is changed, very probably the LD_{50} will change. Consequently, any comparisons between compounds should be made only when the same route of administration is involved.

Even though the LD_{50} obtained in a given laboratory may be calculated to be a rather specific number, it is well recognized that different laboratories obtain different values for the same compound in the same species when the routes of administration are the same in both laboratories. In one study of interlaboratory variation in LD_{50}'s, the authors concluded that, although different laboratories obtained different results, none of the absolute differences were great enough to change the conclusion regarding the relative hazard of the chemicals that were tested.

During the experimental procedure that was followed in order to obtain data for the LD_{50}, a number of additional items of information pertinent to an understanding of the biological effects of any compound are acquired. For example, some of the animals that received a low nonlethal dose of the compound may have shown no effect. The difference between this apparent no-effect dose and the lethal dose gives the investigators information on the range of safety of the compound. In other words, if this difference is small, then the range of safety is small. If the difference is large, then the range of safety is large. In the case of a compound that is going to be used as a drug for the treatment of some illness, it is common practice to determine the quantity of the agent that is necessary to produce the therapeutic effect (i.e., ED_{50}, or effective dose for 50 percent of the test animals) in the same manner that the LD_{50} is determined. It is apparent that the quantitative differences between the LD_{50}'s and ED_{50}'s is an index of the magnitude of the safety of the use of a drug for the treatment of an illness. For nondrug chemicals, a good index of the safety of the chemical would be (1) the magnitude of the dose necessary to produce harm (i.e., the LD_{50}) as compared to the dose that can be administered without producing an observable effect (i.e., NED_{50}, or no apparent effect dose). The procedure for determining the LD_{50} of a chemical thus serves to supply a considerable amount of information in addition to the single number identified as the LD_{50}.

One of the objectives of this symposium is to consider the future of animals as one system in research and development, as well as in education and testing. I have just described a basic toxicity test that involves the use of animals. Usually the only data that are generally made available following the conduct of this test is the LD_{50}. If this data is not worthwhile, then in the future the test should not be done, and this answers the question of the future of animals in at least this test. On the other hand, I believe that the data, if properly and completely reported, are not only worthwhile but are essential if we are going to use increasing numbers of chemical agents for their beneficial value to man. I say this not because we absolutely need the figure described as the LD_{50} of a compound, but rather because we need all of the information derived from the acute-toxicity test.

In my opinion, the harmfulness or safeness of a compound cannot be determined through the establishment of any single figure (such as the LD_{50}). There are, of course, some outstanding exceptions to this statement, among which, for example, would be some of the extremely toxic pesticides of the organophosphate type. As a rule very few compounds possess the property of such extreme potency. Rather, for the large numbers of compounds that are available, the LD_{50} should be viewed as a "ball park" type of figure that is subject to change depending on the type of conditions that are existent at the time of use of the chemical. Thus, anyone who considers the LD_{50} as some sort of exacting figure to which is attached a "divine aura" about the toxicity of a compound is not well informed. Rather, the LD_{50} is a figure acquired by the use of valid scientific methodology that identifies the order of lethality of a compound under a specific set of circumstances, and in this sense it is very useful for purposes of comparative evaluation with other compounds that may serve similar economic purposes.

Therefore, although the LD_{50} is a basic time-honored "standard," the figure per se in most cases is only of casual interest as far as understanding the toxicity of a compound is concerned. It is a measure of potency and not a measure of toxicity. It is the information that is obtained in the process of determining the LD_{50} and the slope of the lethality curve that is a measure of toxicity of a compound. The current state of our knowledge about all of the factors that influence the acute toxicity of chemical agents are so evasive that the artificial system has yet to be devised that can substitute for the whole-animal toxicity test.

I have described what is probably the most basic, time-honored, and simple of all toxicologic tests, i.e., the determination of the acute short-term toxicity of a chemical agent and the significance of the findings. For comparison purposes, let us look at a more complicated type of longer-term biologic test. An an example, I have selected a test that is used to determine reproduction. Tests designed specifically for the detection of effects of chemical agents on reproduction are rather new, having received general recognition only in the past 10 years.

Tests for effects on reproduction have as their final objective an estimation of effects on fertility, on gestation, and on the offspring. Thus, there are three segments to any reproduction toxicity test. Effects of compounds on fertility are reflected by toxicity in the parent male or female or both and may be the direct result of altered gonadal function, estrus cycles, mating behavior, and conception rates. In addition, effects on fertility will reflect any adverse effect on implantation of the fertilized ovum. The second segment of the test is concerned with the development of the fetus, its degree of normality, including teratogenic and mutagenic effects, and intrauterine mortality. The last segment of the test is concerned with effects on the mother, such as effects on lactation and acceptance of the offspring, and with the offspring with respect to its growth development and sexual maturation. The result is an overall evaluation of the toxicity of the test compound on multiple systems within the animals when the agent is given to the animals over a prolonged period of time. Figure 1 is a schematic presentation of Friedman's three-generation type of study for effects on reproduction.

In the figure the F_0 animals (rats) are maintained after weaning until the females reach a weight of 180 g and the males 275 g. In the figure this is identified as the growth period. The time required for growth is approximately 5 weeks but will vary with different strains of rats. During this time the animals are maintained in groups, each of which consists of a minimum of 10 male and 20 female animals with males and females caged separately. Control and treated groups are included in the study. The test compound is incorporated in the diet or drinking water. During the last 2 weeks the estrus cycles are followed in the females, and at the end of the 5 weeks the females are exposed to the males. The occurrence of copulation is established by daily examination of vaginal fluid for sperm, and this finding is considered day 0 of pregnancy. Pregnant females are then housed separately in individual cages. Dosing of the test animals is continued, and the vaginal fluid examinations are continued to check for the absence of new estrus periods in order to further establish that pregnancy had occurred on

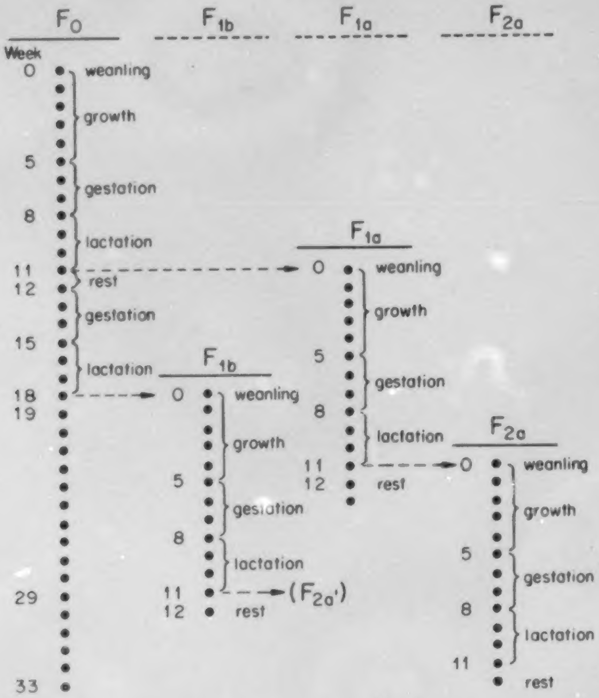
F0
F1b
F1a
F2a
Week
0
weanling
growth
5
gestation
8
lactation
11
12
rest
gestation
15
lactation
18
19
29
33
F1b
0
weanling
growth
5
gestation
8
lactation
11
12
rest
(F2a')
F1a
0
weanling
growth
5
gestation
8
lactation
11
12
rest
F2a
0
weanling
growth
5
gestation
8
lactation
11
rest

FIGURE 1 Generations and time intervals involved in a three-generation study of the effects of chemicals on the reproduction process in rats. Courtesy, *Essentials of Toxicology*, Lea & Febiger, Philadelphia.

day 0. Near the end of the period of organogenesis, on day 13, half of the females from both control and test groups are sacrificed and examined for number and distribution of embryos, empty implantation sites, and embryos undergoing resorption. At the same time a complete autopsy is performed, and histological sections are made of all lesions that are observed.

The remaining animals are continued as test and control animals and allowed to litter normally. The duration of gestation is calculated (approximately 21 days), and the animals are observed at delivery time. The live-born and stillborn pups of the F_{fa},generation are counted, weighed, and examined for abnormalities. The F_0 generation of animals is continued on the test and control diets while nursing the pups. The F_0

animals have now produced and nursed their first litter in 12 to 14 weeks, and data have been obtained on their fertility, pregnancy, parturition, and lactation. After 1 week rest, they may be remated for the initiation of an F_{1b} generation.

The F_{1a} pups have been exposed continuously to the test compound during embryogenesis, fetal development, and lactation. When the F_{1a} pups are weaned, the test diet is started on this generation, and the animals are followed for growth for 5 weeks. At this time they are tested for reproductive performance as in the previous F_0 generation. The same procedure as used in the F_0 generation is followed so that identical observations can be made on the F_{2a} generation.

All animals carried through reproduction studies should eventually be sacrificed and subjected to complete gross and microscopic pathological examination. All stillborn pups should also be examined for skeletal abnormalities. The three-generation reproductive study will supply data on accumulation of test compounds regarding all types of toxicologic tests and particularly for effect on reproduction. Reproduction studies that include determination of the reproductive capacity of the F_{1b} animals can be performed in a total of 30 to 36 weeks.

As observed with other types of toxicity, it appears that there are both strongly and weakly acting compounds in regard to effects on reproduction. Furthermore, the spontaneous occurrence in control animals of stillborn pups and teratogenic effects on pups necessitates that evaluation of the data be subjected to rigorous statistical procedures. Strongly acting compounds can readily be demonstrated to produce significant effects. Accurate data on weakly acting compounds necessitate careful conduct of the test procedures on adequate numbers of animals. The valuation of the absence of an effect on reproduction involves a probability evaluation and the exercise of scientific judgment in interpretation of the results obtained.

A properly conducted reproduction type of experiment employing an adequate number of animals and at least three different test doses, whereby the largest dose represents a nearly maximum tolerated dose and produces no significant effect, represents the degree of perfection that is currently obtainable in experimental toxicology for determination of the safety of a compound.

At the present time very little information is available regarding just what factors are going to determine whether or not a chemical agent will have a significant effect on a complex series of biological events, such as those involved in the process of reproduction. Therefore, I would be at a loss to evaluate a compound for its effect on reproduction by any method other than that which involves the actual animal experiment.

Currently and for some extended time in the future, it is very probable that it will continue to be desirable to know if a new compound possesses any capability as a pharmacological agent and what its harmful actions may be. Although there is some potential for use of tests other than whole-animal tests for some forms of toxicity, it will be necessary to expose experimental animals to the compound under the conditions of a suitable whole-animal test program that is capable of detecting the actions of the compound. Even if all effort at attempts to develop new drugs comes to a halt because of the prohibitively expensive tests that are required, it would still be necessary to know how harmful or how safe a new chemical compound may be for the human who is going to use it or even be exposed to it. Thus, it will be necessary to expose experimental animals to the compound under the conditions of suitable test programs that will evaluate the toxicologic potential of the compound.

DISCUSSION

KOURY: How do you statistically determine how many animals it takes to do a proper LD_{50} or any other toxicological test, and is there not the possibility statistically of reducing that number?

LOOMIS: The number of animals that are going to be needed in a given test depends on how much information you already have about the compound. If you start with a compound on which you have no information, then you have to do these preliminary dose-finding studies to a level where you can get some confidence that you are going to be able to be in the range of measurable lethality with subsequent tests.

The number of animals needed in the final test can vary, depending usually upon the individual variation in the animals. Probably the minimum is 4 in any given group; the maximum is 10 in any given group.

BRAUN: Dr. Loomis said that what will stimulate further efforts to find a substitute for the use of the whole animal is to create a problem that cannot be solved by using animals as they are used today. I think we have that problem already. We have a problem that can't be solved, and the fact that we are all here today shows that there is an increasing awareness of it.

The problem is increasing concern about the volume of sacrifice of animal life. I think as more is found out about animals, and we are beginning to discover that some primates can almost communicate in a kind of a language, there is more interest in the scientific community to find these alternatives. The problem that cannot be solved by using the whole animal is that we don't want to destroy endlessly and in unlimited numbers the animal life that we share this planet with.

LOOMIS: I understand your point very well. I made the statement primarily with respect to the fact that there are certain problems in biology that are prohibitive to approach from the standpoint of whole-animal studies at the present time. They can be approached from studies not involving whole animals, and wherever this is feasible, this type of approach should be taken. I have given one example, and that is the studies in mutagenesis.

HANLEY: You mentioned some tests that are given for the lifetime of the animal.

Among those animals was the dog, where the test could possibly go on for 7 years. Would this be an example of the problem of the laboratory dogs that are kept for long periods of time in cages? This subject has been brought up a few times during this symposium, and everyone seems to be interested in it. Could that dog stay in a cage for 7 years?

LOOMIS: Yes, dogs have been kept in cages for longer periods than that. Actually, I think, wherever long-term toxicity tests are conducted in dogs, there is a direct effort made to exercise these dogs periodically, so they really don't end up continuously being in cages. However, they may be for several weeks at a time.

KOURY: Is it reasonable to keep a dog in a cage for several weeks at a time? That is a matter of opinion clearly. I spoke with a gentleman yesterday. When I complained to him that I was very distressed that we couldn't get the Agriculture Department to require 20 minutes of exercise for a dog every day, he told me that he takes his dog out once a day for the necessities and then brings it in. The dog never moves from the bottom of his bed, and therefore we don't have to worry about the problem.

But there are others of us who wouldn't treat our dogs that way. Does it affect in any way the toxicology studies if you don't or if you do exercise the dogs? Assuming that it does not affect the tests, can we not at some point discuss what many of us are very concerned about, which are just basic decencies?

LOOMIS: You mentioned a point on which there are no data. I don't know that you can say on the basis of any study that I know of that the absence of an exercise period was detrimental to the dog. I can say in my own experience in our laboratory, my dogs are let loose in the laboratory. They follow me around for sometimes 3 or 4 hours. I have every bit of concern about my dogs.

On the other hand, there are certain other dogs that will just never become friendly to me. I don't know why, and they are not given exercise.

HARRY C. ROWSELL

The Ethics of Biomedical Experimentation

ETHICAL CONSIDERATIONS

The Shorter Oxford Dictionary on Historical Principles lists several definitions of the term *ethics*, but the most applicable describes it as "the science of human duty in its widest extent" (6). In selecting this definition, we may apply it as meaning human duty or responsibility toward animals. Thus, we are talking about attitudes and behavior, or conscience, if you will. It is recognized that the ethics of a society vary according to increased technological and social complexity. In simple societies it may mean merely obedience to authority. Often, as a society matures, its ethics become more sensitive. For example, following the Second World War, the Western world was so intent on improving living standards that the effects of technology on the environment had little significance. Animals were exploited and species became rare and, in some cases, depleted almost to the point of extinction. It was not until the late sixties and seventies that man recognized the need for examination of technology and its effect upon the ecological balance. This new awareness and concern represented a change in values of society.

Today's society has changed its attitudes toward the exploitation of animals in all areas. In zoological parks and gardens, for example, it was previously considered acceptable to display animals as caged specimens. Today there are those in the general public who feel that animals should be given an environment that provides the requirements for normal behavioral and physiological needs. Also, there is increasing interest and concern about the exploitation of animals in various sports or in circuses. In today's society, some ask if humans have the moral right to wear animal skins or furs.

There is no question that society establishes its own moral values and thus its ethical concerns. It is not difficult for most scientists to develop a rational argument that accepts that, following the requirements of human duty, the use of animals in biomedical research has been ethical. Conversely, it is equally easy for those with opposite views to develop a rational statement condemning the use of animals in biomedical research as a failure to apply human duty.

It is obvious that acceptance of a polarized point of view by any segment of society does not result in satisfaction. Therefore, we must find intelligent, rather than emotional, solutions.

During and immediately following the Second World War, in studies involving animals, the use of the Noble–Collip drum or the Blalock press was accepted by the scientific community as a method to study traumatic injury and the resultant shock. During the war period, traumatic damage to human beings, resulting in multiple fractures, muscle damage, and shock, occurred with great regularity. The argument concerning the use of the Noble–Collip drum revolves around whether or not we accept that, as it applies to animals in experimentation, "the end justifies the means."

Although the Noble–Collip drum was invented by Canadian scientists, this author has never seen one in 25 years of visiting laboratories in Canada.

Some may argue that ethical conduct rests on what helps the individual animal that is involved, not on justification of the results because they may possibly benefit many others. Acceptance of this definition of ethical conduct of fulfilling human duty in its widest extent would result in calamity for animals and for man. It would terminate biomedical studies and their contributions to an improvement in the quality of life, which has been so widely discussed at this symposium. Acceptance of such limitations would also terminate many of the uses of animals by society today. For example, killing an animal for meat, to provide nutrition for human beings and carnivorous animals, would have to end, because the animal that was killed did not benefit. A concerned member of an animal welfare society (4), a practicing vegetarian, said during a discussion of humane slaughter of domestic animals and humane methods for euthanasia of unwanted dogs and cats that, in his opinion, he did not believe that we *could* have a humane death, particularly when the end result was a dead animal. In other words, as it did not benefit the animal that lost its life, therefore the action was not ethical or acceptable. Humane societies forced to kill unwanted dogs and cats by the action of irresponsible pet owners do not consider this action unethical.

Although noted vegetarians such as Percy B. Shelly, George Bernard Shaw, and Leo Tolstoy would not have agreed, most individuals will accept the need for eating meat in order to provide adequate levels of nutrition. In general, the public demands only that domestic animals be killed humanely. If it is ethical for us to eat the flesh of animals in order to nourish our bodies, is it not equally ethical to use animals to develop knowledge that will lead to an improvement in the well-being of both humans and the animals themselves?

Many discussions concerning the justification for the use of animals, in any field, return to the statement made in Genesis, chapter 1, verse 26: "Then God said: 'Let us make man in our image after our likeness, let him have dominion over the fish of the sea, over the birds of the air and over the cattle and over all the earth and over every creeping thing that creeps upon the earth.'" This dominance of man over animals should not suggest that man does not have responsibilities toward them or that the animals do not have certain humanlike attributes, such as needs, dependencies, and emotions. Man, therefore, has the responsibility for establishing the rights of animals and the means to ensure that those rights are understood by all involved in animal use or care. If man does not accept this responsibility, he must be regarded as a parasite, with all the shame that state implies.

When considering man's responsibility toward the animals he dominates, it is important to remind ourselves of Albert Schweitzer's ethic of "reverence for life." While not precluding animal usage, Schweitzer, in discussing scientists' use of animals stated: "In each individual case they must ask themselves whether there is a real necessity for imposing such a sacrifice upon a living creature" (8).

Therefore, there is no question that one of our most important responsibilities is concern for the animals with which we share this planet. We need to learn more fully how to understand and respect these creatures. Certainly we should control them, and surely we may make use of them. However, we should do these things only after careful thought, for we must learn to avoid abuse or waste. An examination of the use of animals cannot be approached on merely a sentimental basis. We must accept that an animal is an animal and not a human being. Although it has its own dignity, its own way of life, its own behavioral patterns, it is demeaning to attempt to equate man and animal as the same.

There is the need to develop in all human beings a humane code of behavior toward animals, in order to establish and preserve the rights of the latter. We cannot overlook the fact that in many areas, other than research, there is neglect, torture, and destruction of helpless and usually inoffensive animals. These occurrences are widespread and chronic, both historically and in today's society, where wild animals are hunted to extinction, domestic animals are denied their "five freedoms" because of intensive livestock raising, and unwanted companion animals such as dogs and cats are thrown out to suffer disease and death.

Thus it appears correct to conclude that, in some members of society, cruelty to animals is a basic human instinct, only lightly obscured by the veneer of hypocritical attitudes and an occasional "Be Kind To Animals" week.

Unfortunately, there are some intelligent people who give very little thought to animal experimentation but who, on being told that scientists often subject animals to horrendous cruelties, conclude without question that scientists are cruel. Such individuals are misguided. Therefore, it is important that the scientific community present the facts honestly and without constraint, in order to educate those who are indifferent or ill informed concerning the use of animals in research. It is important to emphasize that the prevention of human or animal suffering, through the use of animal-based research, is a worthy objective. It is important to ensure knowledge of the concern of the scientist for the prevention of pain and suffering in experimental animals. This is as mandatory as defining the reasons for animal usage. The actions of an ill-informed or misinformed person, however humane-minded and concerned that individual might be, may produce very unfortunate results. The scientist, however, must ensure that the use of animal resources in research is essential to the solution of the problem, that his treatment of animals is humane, and that principles of conservation are employed. Through research we are improving the health and welfare of human beings and the animals themselves and eliminating diseases that once caused widespread human or animal misery.

RESEARCH

In the biomedical sense, a definition of research could be taken as the investigation or study of an animal where the variables are controlled and the animal, at the end of the experiment, has either died or been killed humanely. This is a conservative definition of research. Therefore, we should look at all the areas that research covers in the broadest terms of the biomedical use of animals. This includes not only classical research, as has been noted, but also the use of the animals in teaching, diagnostic procedures, and testing. It can be relatively easy to defend the use of animals in the strict sense of research, wherein we are seeking knowledge to improve the well-being of both animals and human beings. We may defend their use in teaching as a necessary means of providing exposure of students in professional programs (such as medicine, dentistry, and veterinary medicine) or in imparting knowledge of various living systems to those engaged in the biological sciences.

We may justify the use of animals in testing programs because of a need to determine the safety and efficacy of products. Testing is a demand made by governmental agencies, which, in the pursuit of ensuring safety, often appear to be overzealous in the amount of animal testing required.

Although we may defend animal usage in all categories of research, nevertheless, the scientist must accept the need to analyze and evaluate the effectiveness of any such program.

It is important to remember the wisdom of Abraham Lincoln when he said:

> I am not bound to win, but I am bound to be true. I am not bound to succeed, but I am bound to live up to what life I have. I must stand with anybody that stands right; stand with him while he is right and part with him when he goes wrong.

It is important that we do not accept that any usage of animals in the name of research must be defended if we believe it to be improper. Therefore, using Lincoln's example, we must stand with our colleagues when they are right and part company when they go wrong.

REPLACEMENT, REDUCTION, AND REFINEMENT—THE THREE R'S

These concepts were first enunciated by Russell and Burch in 1959 in their book entitled *The Principles of Humane Experimental Technique* (7).

Humane technique must give priority to conserving the numbers of animals required. Conservation of our laboratory or experimental animals is as important a principle in the experimental situation as it is in the management of our wildlife resources. Therefore, the scientist should not find it repugnant when asked to apply the principles of replacement, reduction, and refinement to his or her experimental studies involving animals.

In applying these principles, it is important to ensure that the animal is not subjected to pain or a situation that may give rise to pain. The investigator must recognize the signs of pain in the animal, which may include escape reactions, vocalization, reflex defecation or urination, bristling of hair, shivering, trembling, or defensive aggression.

Replacement

The three R's demand that the correct model be selected, be it animal, computer simulation, or tissue culture. The aim of selection must be the production of the most meaningful results.

If the model is animal, then the correct animal model must be selected (3). It is not sufficient for the research worker to utilize a rat because this is the animal with which he is most familiar or to use a dog because it is easily handled and responds well to human contact. Additionally, it must be established that using this particular animal model is the only way to achieve the information being sought. If it is not the correct one, then replacement is essential.

Members of the scientific community have been responsible for investigating and developing replacements for animals and for developing valuable animal models. Others in the scientific community who are using animals must recognize the work and activities of their colleagues. They should ensure, when using animals, that there is not a replacement technique or a better animal model. The failure to accept replacement, when available, must be considered irresponsible and deserving of criticism.

Reduction

The reduction in the numbers of animals used is another very important concept to be practiced by the scientist. Accepting that the use of the animal is the only means of obtaining the desired information and that the correct model has been selected, it is then important to conserve the numbers of animals used. Conservation has been referred to as a prime concern throughout this presentation. It should be recognized that an animal is a finite resource.

The strategy on planning and carrying out research is important. We have experienced, in the scientific community of today, a change in attitudes. Individual research has given way to the team approach, in which many disciplines (i.e., pathology, microbiology, physiology, and medicine) collaborate to optimize the value of the results obtained. In this form of strategy, research is well planned, and only sufficient animals to produce valid results are used.

The scientific community has demanded high-quality animals for research, and today many pathogen-free animals are available. There is no question that the introduction of the latter has led to a reduction in numbers of animals needed. That the use of healthy, disease-free animals not only reduces numbers required, but also increases production of meaningful results, should make us aware of the need to breed all animals required for research. Laboratory-bred dogs, cats, and nonhuman primates are especially useful in long-term or chronic studies. However, it is accepted that there are certain species where this practice does not appear feasible at this time. For example, at present, breeding programs for nonhuman primates have not reached the level of production to supply scientists with sufficient animals for their research. This is particularly true in Canada.

Refinement

Finally, we must consider techniques leading to refinement of procedures in which animals are used. Since the days of Claude Bernard, the nineteenth-century French physiologist, rapid advances in refinement of equipment and techniques have developed in all biological disciplines. While we recognize such progress, there is a continuing need for improvement.

Replacement, reduction, and refinement must be recognized as being part of the science of "human duty" in its widest context. Two scientists from countries new to the development of research involving animals, T. Antikatzides of Greece (1) and M. Khemmani (5) of Thailand, recounted the importance of this concept in papers presented at scientific sessions of the sixth ICLA symposium, held in Thessaloniki, Greece, in July 1975. Surely if researchers new to this field recognize this concept, can we, who have had so much experience in this area, ignore it?

PROCUREMENT

Any discussion concerning the ethics of biomedical experimentation involving animals must take into consideration the procurement of animals for research. It is important that all animals used in research be healthy and behaviorally normal. An unhealthy animal or one that is behaviorally deficient cannot provide meaningful results. For example, the procurement and use of nonhuman primates must be based on the judgment of such societies as the International Committee for Laboratory Animals and the International Primatological Society. These groups have urged scientists to contribute to the conservation needs of nonhuman primates by introducing and demanding humane and efficient procedures for their capture, translocation, and maintenance prior to and during use; by recommending that the use of primates as pets be prohibited; and by promoting and development of breeding programs to meet long-term requirements for different species. Their most important recommendation is that scientists be selective in the usage of nonhuman primates by employing other animal models when appropriate and rejecting research on endangered and rare primates that would adversely affect survival in their natural habitats. Every effort must be made for the resources required to develop breeding programs. Again, in order to protect this resource, it is recommended that, when possible, research workers share various tissues and organs or portions of the nonhuman primate.

It has been recognized that dogs and cats obtained from random sources can provide useful information in certain studies. Many are available because of the irresponsibility of pet owners who fail to provide proper care for their "companion" animals. There is, without question, greater cruelty in the streets, where unwanted strays are abandoned, than in the laboratory, to which they may be taken. Nevertheless, the concern and sensitivities of the general public regarding the use of these "pet" species must be appreciated by the scientific community.

It has been claimed that certain humane societies and animal welfare agencies ignore the benefits of research or the improvement of human health and welfare when they refuse to release these unwanted and unclaimed dogs and cats for research purposes (9). However, the scientific community must attempt to understand and appreciate the sensitivities and objectives of a society concerned with the protection of animals.

There should be no justifiable objection by animal welfare agencies to providing scientists with dogs that are anesthetized and not allowed to recover if safeguards are provided that satisfy the requirements of humane societies. Such stringent safeguards should include "open door" policies, for there must be no element of doubt concerning either humane treatment or humane destruction of such animals. Thus, it is difficult to criticize a scientist's request that pounds operated by a humane society provide animals for acute, nonsurvival studies if these animals are to be killed because no home can be found for them. Release of dogs for such studies is practiced in many Canadian communities.

Having supported the acquisition of acute, nonsurvival dogs from pounds operated by humane societies, accepting that this reduces the need to kill a second dog required for the experiment, there remains a need to find an answer concerning the release of dogs for use in long-term or chronic studies. This is an area where it is important for the scientific community to understand clearly the objectives of the humane societies. Although it might appear paradoxical to state, it would be considered improper to release dogs from pounds operated by humane societies for use in long-term or chronic research studies when the release of dogs for acute, nonsurvival studies has been condoned. However, it must be accepted that, during a long-term or chronic study, a painful situation might develop despite all safeguards and controls, because of human failure.

Unfortunately, we have not found a way to absolutely "idiotproof" our care and handling of experimental animals. Therefore, we should not ask the humane society to accept the risk of suffering, which is possible in chronic or long-term investigations.

A humane society should not be asked to compromise its principles of prevention of pain and distress when members of the scientific community are not willing to sacrifice their principles concerning the need for use of animals in research.

PAIN, DISCOMFORT, AND THE EXPERIMENTAL ANIMAL

The care and use of animals for experimental purposes should be based on the principle that pain and discomfort must be avoided. To this end anesthetics and analgesic agents should be employed in an appropriate manner, unless specifically withheld as a requirement of the experiment. Pain-relieving drugs should be continued as long as necessary.

Experiments in which pain and discomfort are an unavoidable consequence should be undertaken only when, on the basis of expert opinion, there are reasonable expectations that such studies will contribute to the ultimate enhancement of the knowledge of life. The degree of pain should never exceed that determined by the humanitarian importance of the problem to be solved by the experimental study.

The ultimate responsibility for the prevention of pain and discomfort in the experimental situation lies with the investigator, who must recognize and institute necessary practices to alleviate any suffering.

It is not only in the experimental situation that pain must be avoided, for animals suffer if improperly housed or handled. It is known that animals respond in a positive manner to gentle handling and considerate attention.

RESPONSIBILITY FOR ANIMAL CARE

We are morally responsible for any living thing that we cause to be dependent upon us. Animals brought into research, teaching, and testing environments are totally dependent on us for their survival. Exemplary standards of humane care and treatment are primarily the responsibility of every person associated with such captive animals. Modern buildings and equipment alone may not result in excellent animal care. Skill, common sense, and concern on the part of the personnel using and caring for the experimental animals are essential. There are certain individuals who have specific responsibilities.

The Scientist

The scientist must select the best method for achievement of meaningful results. When animals are involved in the method chosen, he is completely responsible for their humane treatment and care. He should have knowledge of the behavior, biological characteristics, requirements, and care of the species being utilized. The scientist must be prepared to terminate the experimental study at any stage when, on the basis of his judgment and skill, is continuation is likely to result in unnecessary suffering.

The Chief or Director of Animal Care

It is important that a chief or director of animal care be appointed in every institution using experimental animals. He should be qualified in an appropriate scientific discipline, possess considerable experience with a variety of species of animals, have an understanding of the requirements of research, and be a competent administrator. He should recognize the need for, and be responsible for, establishing educational programs that will improve the quality and efficiency of animal care. It is his responsibility to ensure that research animals are of high quality, appropriate to the requirements of the investigation.

In order to prevent unnecessary distress to the animal, the director must have the authority to prevent unqualified people, whether they be scientist or technician, from handling or using animals.

Support Personnel

The animal attendant, the animal technician, the animal health technician, and the research technician have a part to play in the development and application of ethical attitudes as they relate to the use of animals in research. These staff members are in an excellent position to observe animals on a daily basis and to ensure humane care and treatment. High-quality daily management, observation, and record-keeping are of great importance in making the animal's role in research meaningful. In addition, such practices are essential in the prevention of unnecessary suffering or distress. Support personnel must receive the necessary instructions for provision of immediate assistance to the animals in their charge should an emergency situation arise.

Institutional Responsibility

It is essential that every research establishment where animals are used experimentally recognize its responsibility for control of the care and use of experimental animals by scientists in their research or teaching programs.

It is at the institutional level that controls can best be exerted. Research establishments have an obligation concerning the propriety and the nature of experiments involving animals in their institutions. Their responsibilities may be met by establishing a functional animal care committee. Membership of the committee should be composed primarily of senior scientists with experience in the care and use of animals in teaching, research, or testing. Members of the academic community from faculties or departments not involved in research employing experimental animals may be added in order to lessen the opportunity of biased decision. In some areas, members of the lay public concerned with animal welfare, such as administrators of humane organizations within the community, may be useful additions. In large multidisciplinary institutions, subcommittees may be required to deal with specific areas or problems.

The functions of the committee include making recommendations to and formulating a policy for the research establishment concerning the procurement of animals, animal facilities, animal care and research personnel, and all procedures and techniques involving animals. These should comply with other institutional policies and federal, state, or local laws and regulations.

The animal care committee should not become involved with the scientific merit of the project, but should pass judgment on the ethical aspects of procedures involving animals. Therefore, before research is undertaken, animal care committees should possess all information concerning the numbers and species of animals to be involved and the procedures or techniques proposed.

The animal care committee should be responsible for:

1. Any activities, procedures, and facilities involving animal use.
2. The procedures for ensuring health and comfort of experimental animals.
3. The training and qualifications of animal care personnel.
4. The procedures for prevention of unnecessary pain, including the use of anesthetics and analgesics.
5. The procedures for euthanasia of animals.

The animal care committee, if it believes that proper procedures are not being followed and unnecessary pain is being experienced by the animal, should be empowered:

1. To stop any objectionable procedure.
2. To humanely destroy the animal if procedures cause distress that cannot be alleviated.

Legislation, no matter how comprehensive, can be effective only when there are sufficient numbers of qualified inspectors available to enforce the regulations. Because of the cost and the unavailability of qualified inspectors, legislation is enforced on an irregular basis. On the other hand, the institutional animal care committee can make its presence felt through daily surveillance.

THE PRE-UNIVERSITY USE OF ANIMALS

No discussion on the ethics of the use of animals in biomedical research would be complete without taking a stand on the use of animals in experimentation at the pre-university level. Many organizations have recognized the need to develop regulations governing such experimentation. Present regulations have evolved over years of experience. In the early years of science fairs, there was little regulation, followed by years of increasing restrictions because of an increasing abuse and misuse of animals in science fair projects. The sincerity and motivation of the desire to encourage students in an understanding of life cannot be questioned. However, this presentation has emphasized our responsibility to ensure that animals are used properly and not wasted.

Therefore, we demand that our scientists, before engaging in animal-based research, be successful in an undergraduate university program and undergo additional training in a graduate program, either at the master or doctorate level. Only then, and under the supervision and direction of those experienced in animal research, should they be allowed to perform experiments on animals.

In Canada, abuse and unnecessary suffering of animals has taken place in science fairs. Some young people have developed ill-conceived concepts about scientific investigation. Experience this past year has resulted in Youth Science Foundation acceptance of the recommendations of its Animal Care Committee that the regulations for the use of animals in science fair projects be modified. Important new regulations are:

- If experiments are to be conducted on living subjects for science fair projects, then only lower orders of life may be used. Lower orders such as bacteria, fungi, protozoa, and insects can reveal much basic biological information.
- Vertebrate animals are not to be used in experiments for projects for science fairs, with the following exceptions:

(a) Observations of normal living patterns of wild animals in the free-living state or in zoological parks, gardens, or aquaria.

(b) Observations of normal living patterns of pets, fish, or domestic animals.

(c) No living vertebrate animal shall be displayed in exhibition in science fairs.

Replacement techniques permitting the elimination of animal experiments at this level will not discourage students from a career in the biomedical sciences.

There are many young people growing up today who will never have any exposure to an animal. Therefore, adequately designed and equipped animal care rooms with trained staff in schools and programs have evolved to allow students to understand and to develop sensitivities toward those living creatures with which we share this planet.

SUMMARY AND CONCLUSIONS

There is no question that the humane care and use of all animal life is the ethical duty of a conscientious society and the prime responsibility of those using animals for experimental purposes.

The contributions of biomedical research to human and animal health and welfare, obtained through the use of animals, requires no recitation. However, it is unfortunate that, as Dr. Malcolm Brown, President of the Medical Research Council of Canada, recorded in his annual report of 1973–74 (2):

> The public as a whole, does not often recall the major accomplishments of medical science, the conquest of a large number of infectious diseases. It takes for granted the tremendous changes in the skills of anaesthetists. It has forgotten that pernicious anemia was once truly pernicious. The prevention of mental retardation by the prompt diagnosis of phenylketonuria and the maintenance of health in those who receive replacement hormone therapy are by and large, left without comment save by those directly involved. . . . Many palliative and partially successful treatments have been made possible by research and it has been argued that if it had not been for this research we would not be burdened with so many expensive procedures. It is sometimes claimed that in this sense, research has made things worse and not better. Before it is concluded that things are in fact worse, it should be remembered that few of us will refuse help offered even though that help is imperfect. When we need what even imperfect treatments can offer we grasp for it. In fact, the public demand for these types of services means there is no turning back. The answer obviously lies in the acquisition of knowledge which will permit the replacement of partially successful methods by fully effective methods, and this knowledge can only come from research.

Rational people must be able to see and follow the contributions and the need for animal experimentation and to judge the complexities. Unfortunately, it is a many-faceted and controversial subject provoking violent opinions, including unreasonable ones, on both sides.

Biomedical scientists should not be required to defend themselves as decent, humane individuals. Similarly, those in the animal welfare movement should not need to defend themselves as individuals more interested in animals than in the health and welfare of human beings.

Conflict of opinion can be constructive and may provide answers to complex problems, if zeal for a cause is replaced by temperance rather than blind commitment, with both sides agreeing to cautious evaluation.

In closing, it must be reiterated that the value and contribution of the experimental animal cannot be questioned. The issue is the requirement to practice, in animal experimentation, the concept of replacement, reduction, and refinement. All are consistent with the production of valid, meaningful results that, hopefully, will allow replacement of partially successful methods of disease treatment, in both man and animal, with those that are fully effective. To the three R's we may add Dr. Carol Newton's three S's: good science, good sense, and good sensibilities.

Finally, any discussion on the ethics of biomedical experimentation would be incomplete without making reference to the profession to which I belong, the veterinary profession. This is the only profession having a total commitment to the relief of pain and suffering in animals.

Therefore, veterinarians must provide the lead in all matters relating to animal welfare, which means the total well-being of the animal. Conclusions must be based on hardheaded observations and professional experience. Veterinarians must accept the mantle of responsibility and have the courage of stating their convictions, whether they be popular or unpopular.

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DISCUSSION

CASS: We have no reason to keep animals or any form of life in our laboratories, except as we use them for research, teaching, testing, and the variety of other uses that we purposefully plan. Yet we talk about animal care as a facet separate from research. Tradition among investigators has led them and us—my background is a part of a bench group in toxicology—to say, "Animal care and the animal facilities are down there, and 'research' is in our labs, up here," as though they were two totally separate entities. The fact is that, because laboratory animals are only in our laboratories for the purpose of planned use, the animal subject, its surroundings and the "research" are one.

Being one, it warrants that our budgeting systems recognize it. As investigators, we scream like banshees if we are made to use instruments that aren't sensitive enough to give us the answers we are seeking or to employ technicians that are incompetent and unreliable; and we scream if the research laboratory setting is not proper. However, there is not the same vehemence expressed in regard to the animal and its surroundings, the setting from which investigators first obtain their data and observations. Laboratory animals are living, dynamic, biological entities, and they are the subject of study. They are not the objects. They are not a supply or a tool, as some people allude to them.

I would like to suggest there is little difference between the animal and its surroundings in the laboratory and the experimental situation when compared with the human patient in the hospital. The physician, nurse, and all of the other medical staff can best apply their capabilities, because the total hospital setting provides the kind of maintenance and support that allows them to do the things they know best.

I ask this question: How different is this from the laboratory where the animal subject is being studied? Shouldn't the investigator be able to "do his thing," as he can do it best, in a properly staffed and managed animal study setting, just as a physician does in the hospital setting?

SPIRA: The last speaker mentioned a possible open-door policy. I remember, when there was sort of an open-door policy, people found out what was going on. There was one in Britain recently with the smoking experiment, but they sort of had to give it up. Then there was the beagle experiment that the military people were performing. I think a lot of the laboratory animal experiments depend upon the fact that there are closed doors.

On this discussion on ethics, it was mentioned that people and animals are different. I am aware of that. So are people different, for that matter. There is the wino on the street and the guy who is reading philosophy. There is a similarity between a wino and the researchers here. The animals, whether they are mice or whatever, all feel pain. There seems to be very little concern here and an absolute lack of urgency. A speaker discussed cell culture this morning, and it was really impressive. There was a certain delicacy about it and theoretical underpinning. It was almost a beautiful design. Then there was—I don't know if it is professional courtesy—a mention that it is complementary to using animals.

I think that the one thing that is lacking here is the fact that animals feel suffering, or pleasure, the same as you and I.

ROWSELL: I would agree with the speaker. I hope I presented in a satisfactory manner that this implication was there. Certainly animals feel pain. They have a sense of concern. They have their joys and their unhappy periods, but these are different from the joys and happiness of the wino compared to certain other people. My whole object was to say that they are animals, and that doesn't mean that we abuse or neglect them in any way, shape, or form.

I think we are both talking about the same thing. Your reference to open-door policies has to be agreed upon by those members of the scientific community and those in the animal welfare community. There has to be mutual respect and understanding of one another. There can't be blind commitment and zeal on the part of one, and an attitude of "damn the other." There has to be a need for understanding, and we have to encourage it.

I am afraid that there are some people who would want to have an open-door policy to see how they could criticize. They could not assist the animal, but be so critical that it would make it very difficult for the researchers. There has to be understanding on the part of the animal welfare people, as well as the scientific community.

In Canada, we are very fortunate. At my own university, in Guelph and Saskatoon, we have always had an open-door policy with the Humane Society. They can come at any time. Next week I will be showing two very active antivivisectionists our animal facility at the University of Ottawa. I will discuss with them their opinions very freely.

This is not to say that I agree with their opinions, but I certainly have a great deal of respect for them. I know they have respect for my opinions. When we can develop that kind of respect, we develop a general consensus, not one of polarizing the various groups.

GREENSTEIN: I just wanted to comment on the remarks that we don't have concern for pain. I have just come from an institution where I was responsible for the humane treatment of animals. One of my duties was to investigate the protocols before they were begun, to advise investigators as to the use of anesthetics, and to operate as a clinical veterinarian in general. The staff contained four other veterinarians who didn't share the exact same responsibilities. I think there is a concern for pain in many institutions. Many institutions are concerned with the humane care of animals. It would be helpful if the humane veterinary or animal research community could convince the powers who control the purse strings to investigate the need for size of cage, exercise, and so on. There have been no hard data as yet as to what we need in the way of space.

I think we all agree that something has to be done about the confinement of animals. What is humane treatment? I don't think anybody has hard data. If we could investigate this, I think we would help the government and everyone come to conclusions.

FORCAN: I am particularly interested in your description of the committee that at times allowed someone from the public to sit in on some decisions as to what type of experiment should be done and possibly even stop certain experiments where procedures were incorrect.

I understand this type of thing also goes on in India. I would like to ask the group here today if there is any possibility from an extension of the meeting we have been having of setting up some type of panel or committee that could review the type of thing you are talking about in Canada? Perhaps we could get together and start talking about these things. Is this something that the government or something that you personally are doing?

ROWSELL: In Canada, we don't have legislation at the national level. We do have legislation in some of the provinces at the provincial level, but the program that we are proposing is a voluntary control program that is overviewed by the Canadian Council on Animal Care. We recommend that institutions consider the appointment of a member from the animal welfare community. In the University of Ottawa, we have the general manager of the Ottawa Humane Society on our animal care committee.

The University of Western Ontario, the University of Manitoba in Winnipeg, and the University of Alberta have an input through the provincial government on an animal welfare committee of a member of the humane movement. I don't think that it is the same thing that you are proposing. We have an overall national committee review the problems of experimentation and protocols. That is another matter.

It is a very, very difficult thing. I think that the experience with the Scientific Advisory Board in the Home Office in Great Britain points out the many problems that the committee faces. During the past 11 years, I believe they have had five meetings. In previous times, they often didn't meet for a 10-year period. It is a very complex subject. I am afraid it is too broad for me to go into as far as this present program is concerned.

PFEIFFER: I would like to thank Dr. Rowsell for an excellent paper. I would also like to comment that we should look also at other ethics of research. Five years ago, I did a study of nonverbal behavior in a prison. I had inmate consent. I was there only a few hours a day, writing down patterns of nonverbal communication, a completely innocuous kind of thing.

Today I could not conduct that research, simply because there has been a lot of controversy involving research in prisons, schools, and other institutions.

The lawyers at these institutions, because it is a controversial area, have advised the administrations simply to blanket out all research.

ORLANS: I have been waiting patiently to hear comments addressed to the future of animals in education. Since "education" appears in the title of this symposium, it is unfortunate that it has been totally ignored by speakers previous to Dr. Rowsell. I am very glad that Dr. Rowsell had this as part of his discussion. Education is an area in which Dr. Rowsell has been deeply involved. I would like to pay tribute to the tremendous work that he has done in Canada to see that young biology students have a sound basis as to what they can and cannot do to animals, to prepare them to become responsible research scientists.

It seems to me this is an area of tremendous importance, and I am sorry that it has not been addressed more fully. Education cannot be overemphasized. We have had a lot of discussion about sensitizing professional scientists to utilize alternatives to painful experimentation. However, unless we take the high school students and the undergraduate students and give them sound training in the ethical boundaries of what they can and cannot do to animals and what justification they must apply before they inflict any pain, we are going to create a population of scientists who do not understand the constraints under which we should operate.

In the United States, we have come a long way from the 1960's, when it was common for high school students to botch up surgical procedures on monkeys. A climax of animal abuse came in 1968 with the notorious case of a high school student who won a prize for blinding sparrows and then starving some of them to death.

Science fairs have had an unpleasant history of abuses to animals. However, some improvement has occurred. But, there is still a long way to go. We still have students attempting work far beyond their comprehension or skills. We still find untrained high school students attempting skin grafting, toxicological studies on pregnant animals to produce deformed or dead offspring, and other such exercises that are quite inappropriate for this age level.

I would like to ask Dr. Rowsell what steps he thinks should be taken to develop courses for both high school and undergraduate levels to improve the teaching of bioethics with respect to animal use. We have some courses already in medical schools in the United States dealing with the bioethics of human experimentation. However, I have seen very little attention given to teaching young students the ethics of animal experimentation.

ROWSELL: I think, Dr. Orlans, one has to have the support of the teaching profession. You have to have the support of those agencies that are involved in such programs as science fairs. I do think there is a great need to have a proper explanation in schools, both at the primary and at the secondary level, of the responsibilities young people have to the animal community.

We have many major problems and there are many young people growing up today who will never have any exposure to an animal. We do have the responsibility to see good animal care rooms in schools. I despise the practice of keeping animals in the back of the classroom, where students are taught to care for them and to understand their normal behavior and functions. This nonsensical experimentation is well beyond their comprehension; get them to understand and have sensitivities toward those living creatures with whom we share this planet.

LEE M. TALBOT

Ecological Considerations in the Use of Wild Animals for Biomedical Research

INTRODUCTION

Significant contributions to human health and welfare have resulted from the use of wild animals in biomedical research. These values have been abundantly documented in the literature and in preceding papers in this volume, as have the humane and ethical considerations associated with such animal usage. The purpose of this paper is to review the ecological considerations involved when wild animals are used for biomedical research.

The use of animals in research has increased greatly since the Second World War. In the United States alone, several billions of animals are now consumed annually in research. Most of these are insects, although some 50 to 100 million are mammals, of which about 97 percent are commercially bred rodents (9). In 1972, excluding mice and rats, roughly 1.6 million mammals were used in biomedical research in this country, and this total was composed of 54 percent guinea pigs and hamsters, 24 percent rabbits, 12 percent dogs, 5 percent cats, 3 percent primates, and 2 percent "other" (animals from the wild and hoofed stock) (3).

For the purpose of this paper, the term *wild animals* refers to individuals of nondomestic species normally found in the wild. Since primates are regarded as the most important such group, numerically and otherwise, most of the following comments refer to them. However, the ecological principles involved apply equally to any form of wild animal.

In the past, ecological factors have received little attention in the context of biomedical research. Recently, however, there has been increasing concern with ecology and conservation because of the sharp reduction in availability of primates. Illustrative of this trend is *Nonhuman Primates: Usage and Availability for Biomedical Programs*, a comprehensive report of the Committee on Conservation of Nonhuman Primates of the Institute of Laboratory Animal Resources that cites over 200 references (3).

TRADE IN PRIMATES

Primate imports to the United States have fallen steadily since the peak period of imports of the late 1950's and early 1960's. Between 1964 and 1973, there was a 32 percent decrease in primate exports to the United States from tropical countries (3), and between 1973 and 1974, there was a further 33 percent drop (7). India, Peru, and Colombia were responsible for 78 percent of the worldwide primate exports in 1973. However, in that year India reduced her total export quota of rhesus macaques, her primary primate export to the United States, to 30,000. Peru and Colombia made an even more drastic cut. In 1972, these two countries had supplied the United States with 43,400 monkeys, more than twice as many as India and more than half the total U.S. imports. In 1974, this figure was reduced to 4,600 (9).

The trade in wild animals for biomedical research, like any other trade, is a process that involves a "supply end" and a "demand end." The supply end is usually a tropical or subtropical country where the animals are captured and exported. The demand end is usually a developed country in a temperate zone where the research facility is located and into which the animals are imported.

Given the sharp reductions in supply, most considerations of ecological impacts has been directed to the supply end of the process, and most specifically to the depletion of that supply. This is quite appropriate. There are, however, potentially significant ecological impacts at the demand end.

ECOLOGICAL CONSIDERATIONS AT THE DEMAND END OF THE TRADE

The matter of greatest ecological concern at the demand end involves the potential consequences if the animal itself, or diseases or parasites it carries, should escape and become established in the new environment. The potential ecological impacts from introduced exotic species have been well documented in a large number of cases. Introduced exotics disrupt indigenous ecosystems in a variety of ways, ranging from direct predation to competition for food, water, shelter, and living space.

Diseases and parasites introduced by exotics can have a devastating effect on indigenous wildlife, crops, and livestock and on human health. These dangers are so acute and so well documented in the United States that the Department of the Interior has felt it necessary to revise its regulations for importation of exotic species, in effect excluding all but those few species that have proven harmless (1, 12).

Because individual primates are usually quite expensive, research facilities utilizing them are sufficiently well secured so that escapes are rare. The pet trade, therefore, is regarded as the source of much of the existing problem. However, in many cases the same dealers import animals for both research and pet purposes, and it is difficult to distinguish whose animals have escaped. Since most primates come from the tropics, it is only the more tropical portions of the United States that would be vulnerable to colonization by escaped primates. However, where the conditions are appropriate, this has happened. For example, there are populations of squirrel monkeys now established in Florida.

The other side of this coin is the impact of the temperate-zone environment, including the laboratory facility itself, on the species that has been introduced. New diseases, parasites, and climate, including such factors as day length and ranges of temperature and humidity, are only a few of the many ecological factors in a laboratory situation that differ from the imported animal's original habitat.

Restrictions on social and behavioral activities may play an even more important role. This is particularly so since most primates are "*k*-selected" species, which, as discussed below, are adapted to a stable "climax-type" environment and are relatively intolerant of conditions that differ. The consequences of these ecological differences may be manifested in several ways that affect the purposes for which the animal was captured. These can include changes in state of health, growth rates, reproductive potential, and behavior, and there may even be physiological alterations. These factors become especially significant when one is considering captive breeding programs.

In view of the reduced supplies and allied factors, there have been a number of suggestions for development of breeding centers and even research facilities in the tropical areas in or near the animals' original habitats. While there are advantages to this approach, particular care must be taken to avoid the danger of accidental introductions into those environments. This potential problem is much more acute than in the present temperate-zone situations. The tropical environment is suitable for the primates, so that any escapees have a better chance of becoming established. There are likely to be indigenous primates that could be adversely impacted by such introductions and that could readily spread pathogens received from the escapees.

ECOLOGICAL CONSIDERATIONS AT THE SUPPLY END OF THE TRADE

The primary ecological considerations in wild animal usage are at the supply end. These considerations involve the capture and removal of the animals from their habitat, and there are three main classes of ecological impacts involved: depletion of the species captured, impact of that depletion on the ecosystem, and additional impacts of the capture methods involved.

Capture for the biomedical trade has been a major factor in the gross depletion of primates throughout much of the world. In India, during a 6-year period in the 1950's, over 1½ million rhesus macaques were exported. Although precise data are not available prior to this period of capture, there were an estimated 20 million macaques in the state of Uttar Pradesh alone. After that capture, only an estimated million remained (9). While adequate figures are not available, it appears that this pattern has occurred throughout primate habitats wherever there was a significant harvest for export. The reductions in exports, noted above and detailed in the report of the Institute of Laboratory Animal Resources, have been attributed to reductions in supply, not reductions in demand (3).

The Philippines provide another striking example. Exports of wildlife, primarily primates, reached a peak in 1959–1960. Only 3 years later, the exports had dropped to 8 percent of that peak (11). At that time, in the process of a resource survey throughout the Philippines, I only encountered monkeys twice during 6 weeks spent on foot throughout much of the best primate habitats of the islands.

However, the gross depletion of primates has not resulted solely from the biomedical trade. The Indian estimates illustrate this well, with a 6-year export of 1½ million animals, coinciding with a reduction of population of an estimated 19 million animals. One factor involved is the wastage of animals in the process of capture and shipment (3). Estimates vary, but high losses at all stages in the capture, handling, and shipment of primates have been documented. Perry (9) notes:

> No one knows how many die in the course of capture and transportation. While many observers have seen parts of the system at work, or even followed the entire sequence, mortality varies from batch to batch. Some have estimated a 75 to 85 percent mortality. The lowest estimate I have seen is one-third. A dealer's "normal" losses may be 10 to 20 percent, but an occasional epidemic may cause a near-total wipeout.

Consequently, whatever the true figures, the actual exports from a supply country and imports into a demand one clearly represent only a fraction of the total animals removed.

This situation is aggravated in the case of the larger primates, such as gorillas and orangutans, where it is the young that are captured, often through the process of killing the mother and other associated adults.

However, even these additional direct sources of mortality related to the biomedical take do not account for the total depletion, which is the result of a series of interacting factors. In addition to the biomedical demands, the most important factors are other sources of direct mortality and habitat change.

Primates are often regarded as agricultural pests. The expansion of agriculture into primate habitats, particularly in Africa, has resulted in the killing of large numbers of primates to protect the crops. Primates are also a source of food for humans. Presumably, this was not a factor in the Indian situation, but in other areas, particularly in Latin America, food hunting probably removes more primates than the export trade (8). Capture for the pet trade is another factor severely affecting certain species.

Habitat change, however, is the most imortant single factor affecting primates. Most of the depleted primates are tropical forest species. On a worldwide basis, tropical forests are regarded as the biome that is being reduced most rapidly. Such large areas are being lost each year that, if the present rate of destruction continues, tropical forests would be gone by the year 2000 (10).

In addition to direct removal of forests, road and path construction, incidental to exploration, mining, and other activities, has the effect of making much more of the forest accessible to hunters—i.e., of further reducing the refuge areas for wild primates. Other forms of change, such as removal of "weed trees" in association with lumbering or agriculture, further impact the habitat.

Total loss of habitat will result in total loss of the species that depended upon it, regardless of any additional sources of mortality. However, in general, the habitat loss has interacted with the hunting pressures—hunting for biomedical purposes, pets, or food. Opening forest areas has made the surviving primates more accessible and vulnerable to capture or killing. As the formerly large blocks of habitat are fragmented, the surviving primate populations are isolated in the fragments. These are more vulnerable, and when they are removed or reduced beyond the level at which the population will be self-sustaining, they are lost, because there is no source of resupply. Consequently, an overall level of harvest of primates, which could be sustained in a healthy habitat, results in loss or gross depletion of the population in a reduced and fragmented habitat.

In some cases the collection process itself further aggravates the situation, through further direct destruction of habitat. Some capture methods involve cutting down substantial numbers of trees to isolate a group or family of monkeys in a single tree where they can be captured more easily. In areas of already restricted habitat, the process can further reduce the carrying capacity of the habitat for the survivors.

From an ecological point of view, while capture for biomedical research is not the sole cause of depletion of primate populations, it is an important contributing factor wherever it exists, and it can be a major factor, depending on the species and situation. If the demand continues, the impact on the remaining primates becomes progressively more severe as the habitat becomes progressively more fragmented. Therefore, biomedical demand can be a factor contributing to the endangerment, and possibly the extermination of, species.

LOOKING AHEAD

Looking ahead, and taking the above ecological considerations into account, it seems clear that the supplies of primates from the wild will continue to be reduced. The best prospects for survival of viable populations of wild primates are in some type of reserved areas where their habitats are protected. Existing protection, however, appears grossly insufficient to assure continued viability of all wild primates (3–5). Indeed, from the standpoint of biomedical needs, on the basis of species and continents, existing protection is inversely proportional to demand. The species and areas most needed by biomedical research are those least protected by forest reserves.

However, even with greatly expanded habitat reserves, it is doubtful that any significant percentage of the biomedical needs could be met from populations in these areas. In the first place, such harvest would be in conflict with the basic purpose of many of the reserves. Ecologically more significant, there is no assurance that many wild populations in such reserves could sustain any significant harvest, even if the collection methods could be developed that did not in themselves adversely impact the habitat and the behavior of the animals involved.

In general, the most productive lands for human purposes are also most productive for wildlife (6). Parks and reserves are generally established in the least-productive areas because of minimal conflict with other land use. Consequently, the productive potential for primates, as well as other wildlife, is limited at the start.

Further, most reserves are relatively small, and there is question as to what is the minimum area needed to sustain a heterogeneous gene pool in the primate population. Where the population is reduced to relic fragments, polymorphism—the genetic variability that allows adaptation to changing habitat conditions—is markedly reduced. This appears to be true already with several species of primates, where polymorphism is greatly reduced and vulnerability of the species greatly increased (3).

From the standpoint of harvest itself, most management—where there has been any management—has been based on the concept of a harvestable surplus, usually in the form of maximum sustainable yield (MSY). This concept of management is aimed at achieving the highest sustained yield of a population without reducing the stability of the base population. It was developed on the basis of species known as "*r*-selected," i.e., those with a high reproductive potential and generally flexible habitat requirements. Under some conditions such species produce an annual surplus that may be harvested. However, the validity of the MSY type of assumptions has been seriously questioned, and a recent study found MSY and other ecologically simplistic management concepts inappropriate as a basis for or goal of management (2).

Further, at least the larger primates are not those "*r*-selected" types. They are "*k*-selected" species, i.e., long-lived animals with a low reproductive capacity, adapted to and relying on stable, or "climax," vegetation types. Since virtually all human land use creates successional vegetational stages, the "*k*-selected" species are especially vulnerable to human activity. In any event, such species have virtually no potential surplus population that could be available for harvest.

Consequently, self-sustaining, captive-breeding populations would appear to offer the only real hope for a sustained supply of primates. However, in the past, with the reliance and single-minded focus on wild-caught supplies, there was relatively little research on reproductive needs or methods and little effort expended to develop captive-breeding centers. Consequently, present efforts are totally inadequate to provide what the biomedical profession believes its needs to be. In 1973, captive births comprised less than 5 percent of that year's demand for primates (3).

CONCLUSIONS

In conclusion, it must be recognized that the opportunities for supplies of wild captured animals at the past high levels are gone. In the future, most wild primate populations will make little or no contribution to the routine needs of the biomedical community, beyond the occasional provision of individuals to augment captive-breeding populations.

As a result, it is clear that the biomedical community must do several things: It must lower its demands for primates, it must concentrate its resources on the research and development necessary to establish self-sustaining breeding populations, and it must assure that effective conservation measures are undertaken so that adequate areas of the wild habitats are protected to assure the continued viability of the various primate species in the wild.

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DISCUSSION

ROWSELL: I would like to make a comment and ask Dr. Talbot his views concerning MSY. I think one of the great problems is that biologists really can't tell us what the population dynamics of a particular species might be, and often the method of estimating populations is completely inaccurate. I know that many of our wildlife conservation programs are designed on this very subject, and often they are developed by industry without very much attempt at determining what does exist and what is the MSY.

It is very rare to have them take into consideration such things as environmental problems that have been created by man. I wonder whether or not, when this term is used, we really should put very much credence in it, or should we get rid of it entirely?

TALBOT: Your comments are very well taken. I think we should get rid of it entirely. It has been the basis of most managed harvest of wild species pretty well throughout the world. It has become set in many local laws, in the Constitution of Alaska, and in a number of international agreements.

It doesn't work, and ecologically it can't, even if we had the data, which in most cases we don't. As applied, it does not take into account the impact of harvest and other factors affecting the species itself, nor any of the ecological interactions. Recognizing this, there have been a series of workshops and meetings held in the past year, under the joint sponsorship of a series of organizations, to examine critically this concept and its application. Specialists with worldwide experience were brought in, those who had been involved in the basic research, those who "wrote the books" on MSY, and those who had been involved in trying to apply it.

Their ultimate and unanimous conclusion was that alone it is not appropriate as a basis of management. We must go a great deal farther and manage things from the point of view of the ecosystem as a whole.

HANLEY: In my capacity as coordinator for the San Francisco area, we cooperate with a group called the International Primate Protection League (IPPL), which is based in Bangkok, Thailand. They try to keep track of the demands, primarily for research primates, out of Thailand and all over the world. The IPPL tries to keep track of the demands for the primates and how much exporting occurs, say from Thailand. Gibbons, for example, are not to be exported out of Thailand, but in some cases they have gone out illegally. The IPPL tries to investigate these cases. Have you found any illegalties or any smuggling of primates in your work?

TALBOT: Quite a deal, most of which has been pretty well documented. It still goes on. The problem has been greatly reduced, thanks to the efforts of volunteer groups like the IPPL and the organized efforts of the IUCN.

In this country, trusted recipients are still receiving fliers from places like the Mayfield Kennels and Zoo in Singapore and the Hong Kong Bird Shop. These include, in addition to the regular catalogue, instructions on how illicit things are to be ordered and how they will be sent through. The most infamous perhaps were the ones in which the Hong Kong Bird Shop sent full instructions on how to deal with the orangutans they were shipping in a double box, with pythons in the front part of the box.

Given this kind of a situation, it is clear that it is impossible to police the whole world. The best way to try to do something about it is to get at the demand end, not the supply end. If the demand is cut off, and the U.S. is a major source of the demand, the supply end dries up, because it is too dangerous and not sufficiently remunerative. This is what has happened, for example, to a fair degree with orangutans and some other species.

LADIMER: In this area, I understand that we have adopted a certificate of need requirement, which investigators in the United States are expected to fill out to justify a request for importation of primates and perhaps other animals. Does that work as a good brake or method of control at the demand end?

TALBOT: If it is enforced, I think it is a very good way. That kind of a certificate is part of the system that was set up by the International Convention on Trade in Endangered Species. It depends, however, on the degree of enforcement by the nation at the demand end, as well as those at the other end.

IRVING LADIMER

Root and Branch: Legal Aspects of Biomedical Studies in Man and Other Animals

INTRODUCTION

The inhabitants of our familiar kingdoms—animal, vegetable, and mineral—are all appropriate, indeed necessary, subjects of scientific research. So too are the members of other worlds and systems, known and unknown, in present and future, relevant objects of investigation and study. As populace in the same cosmos, we all share and benefit; thus, at least in a moral sense, we must all contribute to advancement and improvement. Although the interdependencies will vary, interrelationships of some type will continue. Important values and interests will certainly differ, but there is still some common ground in survival. These mutual concerns should be scientifically united as firmly as they were romantically joined by D'Artagnan in Dumas' *The Three Musketeers*, "All for One, One for All."

HUMAN HEALTH AND WELFARE

The essential anthropocentric views traditionally held by professionals and laymen alike directed scientific endeavor to the benefit of mankind. Other living and nonliving members were considered almost wholly in relation to their benefit or worth for human beings directly and indirectly. Even the recent somewhat universal and altruistic concern for all creatures, great and small, is tempered by some presumed general advantage for humankind, if not in a technical or material sense, then in an idealistic way.

Along with this position there was the more significant belief, widely held until the beginning of Darwinian thinking, that human beings as a class were unique—so significantly different from other living beings that, except in a metaphorical way, there was little scientific transferability from lower animals, components, or parts to the whole man. Accordingly, scientific inquiry was largely divided between (1) biological studies to understand plants and animals in terms of their own development and use for human benefit and (2) direct studies and observations on man himself to comprehend illness and health. Biomedical studies were conducted on patients, mainly by clinical analysis and observations of general populations. The early anatomical studies and dissections were performed on human corpses, not always or necessarily preceded by similar work on animals.

By the nineteenth century, however, the analogies among all animals were well appreciated, and the importance of testing human functions on allied species was recognized. Around the turn of this century, in a series of lectures to various medical societies in the New York area, Dr. James Peter Warbasse spoke with intense feeling on the subject, "The Conquest of Disease through Animal Experimentation":

> All animals are so closely linked by the bonds of kinship that information gained from one is applicable to the others. Something can be learned that has a bearing on man, from every living thing. The work which is being carried on by biologists in studying and experimenting in the physiology and pathology of animals is applicable to man. . . . There is a general notion current that animal experimentation means vivisection and that vivisection means the painful mutilation of animals. . . . These notions belong with the traditional horror of the hospital and the madhouse; they are the product of ignorance and prejudice. Practically all experiments performed upon animals are made by medical men or men allied with the collateral sciences; and I know of no class of men who have higher ideals of the humane and who are prompted in their work by more noble motive than they. Almost without exception, these investigations are carried on under the inspiration of helping humanity. (1)

These joined themes of Dr. Warbasse have continued to dominate all discussions of the relationships between animal and human experimentation and have led to the basic legal controls that we have today governing the participation of animal subjects.

Much has been said about the callous treatment and care of animals. I think no one here would or could in good conscience permit or justify cruelty to animals, condone negligence, or any willful or deliberate act that would endanger them in any way. I hope this would be as true regarding people as well as our companion friends.

But I don't see this as an issue for discussion. It is an issue, of course, of great concern. I share that with all of you and hope that we can all do something together to insure that no undue risk or cruelty is imposed on any creature. But the legal significance of the animal–human relationship and continuing development are found in three related aspects, as discussed under the section "Legal Significance" below.

LEGAL INTERVENTION

It is now *de rigueur* to include some observations on legal or ethical aspects in comprehensive discussions of biomedical matters. A generation ago, this feature would hardly have been considered. The explanation probably rests in the public belief that science and scientists have not, despite assurances and recognized dedication to noble and humane motives, shown appropriate concern for people and other living beings. Rather, it is felt that their interests in personal achievement and professional accomplishment have blinded them to the pain of animals and the hazards inflicted on human subjects. Unable to develop and maintain self-regulation, scientists of all kinds are now governed by various legal constraints and moral obligations of almost equal force. Relatively few legislative actions by statute or resolution have served directly to promote or encourage methods of research, with safeguards for scientists against unwarranted charges or complaints. With the support of science through materials and money, there is the expectation that the practitioners will provide their own protection, not for themselves alone, but mainly for their subjects.

Law has become so well entrenched in the scientific milieu that it is difficult to realize that the proliferation of statutes and regulations, federal and state, to control medical research on human beings is the product of about the last 15 years. At the National Conference on the Legal Environment of Medical Science, sponsored by the National Society for Medical Research and the University of Chicago, held in May 1959 (this author was Conference Secretary), the following statement was made:

> The law relating to medical and health research requiring human beings as clinical resource material has developed on a case by case basis. There are no Federal or State statutes directly regulating such research. Where "experimentation" has been used, it has been applied by courts to various types of medical malpractice. (2)

Professor William Curran, in his review of governmental regulation in this field, similarly reports that in the years prior to 1960 there was little "law" on the issues, no federal or state statutes, and no reported court decisions involving liability issues or criminal actions against research organizations or investigators (3). There was even substantial difficulty getting professional recognition of the existence of a potential problem, and the Legal Environment Conference, which may be considered a precursor to this symposium, therefore united three related subjects: animal experimentation, particularly care and use of laboratory animals; legal and ethical aspects of clinical research, particularly amplification of the so-called Nuremberg Code; and anatomical research and education, particularly use of cadavers, autopsy laws, and transplantation. All of these issues are very much with us today, but, although that conference generally proposed ethical control and scientific self-regulation through establishment of standards based on widely accepted principles of social responsibility, the law and its implications have largely prevailed.

ANIMAL EXPERIMENTATION AND THE LAW

In this country, the first attempt to regulate human experimentation appeared in a bill introduced in 1900 for the District of Columbia. It would have specified the methods of study, including prior animal experimentation; types of subjects to be excluded, such as children and pregnant women; qualifications of biomedical scientists; and reporting and approval requirements. This bill appears to have been drafted by humane society members or friends and may have represented an extension of their long-held concern for the safety and protection of animals to human beings. The bill was also supported by groups concerned with use of volunteers as publicized by the yellow fever experiments conducted by the U.S. Army surgeon, Dr. Walter Reed.

Legal involvement in use of animals for research is directly related to the animal welfare and humane movements and the legislation, as well as case law, promoted by their efforts, intended to prevent cruelty to animals. Historically, Anglo-American common law permitted animals, legally obtained, to be used for scientific experimentation and education. Before laboratory and other animals were specifically bred for this purpose, domestic animals, particularly dogs and cats, were used, as well as animals of field and forest. Science employed all types of creatures that were available and appeared to be associated with the disease or condition under study, such as insects, reptiles, and birds, as well as mammals with greater similarities to man. The common law in England has not changed, but the exacting English licensure laws dating back to the last century have established Great Britain as the most restrictive country in its regulation of animal experimentation; these are often recommended by groups in this country as models (4).

Although there is no national restrictive legislation of this type in the

United States, nor anything like the German legislation enacted during the Nazi regime, which prohibited virtually all research on animals, there is a honeycomb of laws, ordinances, regulations, and procedural requirements relating to proper care, shipment, teaching, use, and safety precautions. These apply in addition to ethical codes adopted or endorsed by every scientific organization and professional body using animals. The Canadian self-regulatory program, described in an earlier paper, should be considered for American application before legislation. I refer also to Congressman Foley's remarks, which clearly indicate there is no pressing need for federal controlling legislation at this time, although there is continuing interest.

The legal surveillance in statute and decision stems from two principal, somewhat conflicting, concerns: protection of private property and interest of the State in unclaimed animals and their use. Associated with the latter interest is the State's concern with public education, reflected in legislation regarding vivisection in the teaching of biology and science in primary and secondary schools.

Property Rights

According to John Sembower, former legal counsel to the National Society for Medical Research, courts in this country have held that individuals have only a qualified property right in dogs and cats outside the custody of their owners. Unlike cattle, horses, sheep, hogs, and similar animals so domesticated, cats and dogs without owners "have from time immemorial been considered as holding their lives at the will of the legislature, and properly falling within the police powers of the several states." Under this pronouncement of the U.S. Supreme Court in *Santell* v. *New Orleans and C. R. Co.*, 166 U.S. 698, and a similar decision by the high court of New York, *Fox* v. *Mohawk Hudson River Humane Society*, authorized agencies such as public or nonprofit pounds, humane societies, and shelters may dispose of animals, for authorized purposes, including research, after opportunity to restore lost pets to their owners. In the last decades, there have been numerous so-called pet laws proposed and enacted to prevent use of unclaimed animals, mainly dogs and cats, for research. These laws were based on what Sembower calls the mythical doctrine of presumed intention, that is, that it would be the intent of the owner, known or not, to have such animals killed rather than made available for vivisection. These claims have generally not influenced legislation any more than allegations that disposition of animals to private research institutions constituted improper donation or sale of public property for private use (5).

Although these claims by individuals and organizations have had no significant legal impact, organized and unorganized friends of "man's best friend" continue to object to presumed cruelty in the scientific studies on animals. It is this issue, not the humane disposition of animals that would otherwise be destroyed, that is publicly proclaimed. To answer such arguments, three main programs are under constant development and promotion:

1. Drafting of constructive pound laws for states and other jurisdictions under which unclaimed animals may be donated to recognized licensed scientific or educational institutions, with provision for inspection, environmental regulation, and for proper return of identified animals. Experts in this area are aware that such laws, even if enacted with full public support, require surveillance and education of pound officials and constant demonstration of effective execution of the purpose of the laws.
2. Improvement in number and quality of private sources for laboratory animals in this country and abroad. The resources now required in quantity and the specialized nature of animal and other organisms mandate the specific development of breeding capability and the protection of natural habitats for many varieties. The legal implications here go to the design of appropriate authorizing laws, federal and local, as well as international treaties or compacts, for the care, feeding, protection, and shipment of animals, on the one hand, and the regulation of suppliers, dealers, transporters, and other monitors of such animals, on the other hand.* The U.S. Department of Agriculture, principally as administrator of the Animal Welfare Act and its amendments, and other federal agencies and their state counterparts are mainly responsible for regulation of this type under appropriate statutes. But, in this area, the voluntary codes and regulations of such organizations as the National Society for Medical Research, the Animal Care Panel, and the American Association for Accreditation of Laboratory Animal Care can play a more important role. These laws and codes are intended to assure proper care of the animals and also health protection for handlers and the general public. Such work may obviate the need for further legislative control (6).
3. Professional codes for humane and effective use of laboratory animals. The programs that are likely to have greatest impact are the scientific–administrative codifications and continuing instruction in the maintenance and use of animals for research and teaching. As noted, every responsible scientific group subscribes to such ethical declara-

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*See addendum on Animal Welfare Act, page 313, this volume.

tions, which are designed to insure optimum–minimum use of animals, exposures that are statistically required, painless and careful ministrations and, above all, to dispel even the appearance of exploitation, lack of purpose, or consideration. The dispute in the scientific community and in the public media after the television broadcast of *Primate*, Fred Wiseman's documentary film on the Yerkes laboratory, must be avoided—not by secret research, but by more open sharing with the public of fully developed studies and understandable procedures. For example, the so-called "sex experiments" on cats at the Museum of Natural History in New York brought out pickets against experimentation, as well as publicity and governmental investigation.

Interest of State: Cruelty and Humane Laws

The State's interest in animals, unclaimed and owned, stems from its police power to punish anyone inflicting cruelty and, consistent with this interest, its ability to enact humane laws for protection of animals. Under common law, cruelty included deliberate acts or negligence permitting needless pain, suffering, unjustified death, or destruction. Malice or wickedness was sometimes required to be shown or was imputed because of gross negligence or carelessness.

Obviously, the medical or scientific use of an animal, including sacrifice, is not brought into this definition. And there has been no recorded case of a scientist judged guilty. On the other hand, there have been countless convictions of dealers and suppliers of animals said to be destined for scientific study who have neglected, starved, and even beaten their charges, or permitted them to become sick and diseased. The scientific community cannot be relieved of responsibility, since the suppliers cannot exist without demand—and, in the area of open science, particularly biology, there can be no offense permitted.

The basic control comes through state and local laws that specifically authorize animal experimentation, permit such activity, and limit or regulate it for certain purposes, such as demonstration or education in schools. All states have anticruelty laws or court decisions, and, to permit proper animal experimentation, some have exempted experimentation from such anticruelty laws. In the view of the National Society for Medical Research and similar bodies, such exemptions were well-intentioned, but not needed, since proper experimentation did not involve cruelty.

Even now, there is no consistent body of legislation and regulation in this area, and scientists, teachers, and physicians in many jurisdictions may unwittingly violate some prohibition or limitation. This is particularly true when a community responds forcefully to some presumed callousness to animals, as in a New Jersey town some years ago when a biology teacher was charged with cruel and unnecessary dissection of living creatures or when the monkey sent aloft was said to have been mercilessly damaged.

Effects of Laws on Animal Research

Antivivisectionism, in various forms, has impeded medical research, not only through creating barriers in animal procurement, but also in raising doubts about scientists' motives and capacities. In 1946, the National Society for Medical Research (NSMR) was founded by the Association of American Medical Colleges to educate the public, to combat restrictive legislation, and to devise constructive measures in the use of animals. In many ways, the society was founded for the same purposes as the British Research Defense Society about 50 years before—to demonstrate the values of animal studies. Both later extended their interests to honor human volunteers. The NSMR sponsored the Walter Reed Society in 1952, and it later set up programs to help anatomists obtain cadavers and supported early legal activity toward transplantation and passage of the Uniform Anatomical Gift laws.

Significant as these functions are for the scientific community, the public and many professional groups are apparently not convinced that the capacities and interests of scientists are dedicated to create better research designs. The root problem then is not legislation and regulation to provide more animals or to make it easier to obtain and employ them. No, we have to find more effective and acceptable ways to medical progress with minimal sacrifice of life or risk of pain or damage for any animal, whether high or low. Can the ingenuity of man reduce his need to use fellow creatures without handicap to human health and welfare?

HUMAN EXPERIMENTATION

The conventional formula for using human subjects and animal precedents is well expressed in a recent article by Dr. Joel Bernstein, University of Tennessee Medical Units:

> Although some zealots have argued that much experimentation in humans can be equally well conducted in animals, a primary criterion of usefulness in medical research is that the observed phenomena are reproducible or applicable to human beings. . . . This does not mean that most investigations will be initiated on human subjects without prior

> animal data. On the contrary, for the vast majority of conditions to be studied, thorough animal evaluations, with regard to both safety and potential utility, are a prerequisite to human study. In all cases, adequate animal exposure to determine the safety of a procedure or a therapeutic agent is necessary prior to undertaking human studies . . . there are certain instances where such investigations may have to be undertaken initially or exclusively on human beings. . . . Many disease processes have not been successfully reproduced in animals. (7)

A more specific statement in support of the historical evidence that valid data derived from animal studies have made impressive contributions to public health and that such studies must continue appears in *Nonhuman Primates: Usage and Availability for Biomedical Programs* (8). That report quotes Dr. W. G. Hoag, Director of the Center for Laboratory Animal Resources, Michigan State University:

> The ultimate test of any product for human usage must be made in man himself. The pretesting of such products in animal models is an important step leading to this final test. It is important that the animal model selected provides the test system which simulates the one in the human biological system. This similarity does not necessarily relate to the phylogenetic proximity of the animal species. It is important for investigators in planning animal experiments to look first for the model biological system. (9)

Today, there is an extensive and publicized enterprise in the scientific community devoted to describing available animals for research. For example, we have the series of publications of the Institute of Laboratory Animal Resources, a host of academic and commercial materials, and, more directly related to scientific needs, various research services for supply, maintenance, and development of suitable animal models. The well-equipped service center, such as the National Institutes of Health Division of Research Services, not only develops new models, produces small animals, and maintains facilities for larger stock, but also serves as part of the network of information and exchange in this area and in devising other supports for biomedical research.

LEGAL SIGNIFICANCE

The legal significance of this animal–human relationship and continuing development may be seen, as previously noted, in three related aspects: First, the possible requirements through law or regulation that animal research must, as a matter of compliance, precede human investigations; second, the potential liability for failure to meet scientific standards as well as legal requisites; and, third, the most productive and constructive use of law to serve animal and human welfare. The three basic reasons for use of animals may briefly be summed up as: scientific, economic, and ethicolegal.

Scientific requirements essentially hold that, in view of the many aspects and requisites of research in human health and disease, a wide array of systems and methods utilizing all types of living cultures, organisms, technology, and animals must support human inquiries. Experimental systems for man are often best found in other contexts.

Economic rationales, closely related to the scientific bases, argue that cost–outcome ratios are generally most favorable when we can utilize animals. They can be obtained, supported, and manipulated more expeditiously than human beings, particularly when elements of time, place, and diverse usage are significant. Where rapid growth, large numbers, special varieties, close controls, and management factors are vital, animals must be chosen. This does not, of course, preclude other methods, but I have tried to sum up the cogent reasons for considering animal studies.

Legal and interrelated ethical and social factors are self-evident. Human beings may not be subjected to risk, especially when the studies are not directly beneficial to them, unless and until all other tests and trials are performed. These must include studies on animals when appropriate. Animal studies must be sufficient for scientific analogy or extrapolation, but not inconsiderate or undirected and certainly not excessive in the use of any creature, even the guinea pig, mouse, or rabbit.

Legal Controls

Although statutes exist for the direction or control of biomedical research, such as federal food and drug laws and public health laws, they do not usually specify that animal studies be employed. The Food, Drug and Cosmetic Act, as amended in 1962, does provide, with respect to new drugs, that approval must be based on "substantial evidence" that the product is safe and effective; such evidence consists of "adequate and well controlled investigations, including clinical investigations" by experts "that the drug will have the effect it purports to have under the (prescribed) conditions of use" [Sec. 505 (d)]. Under this provision, the Food and Drug Administration (FDA) has issued both regulations and guidelines for appropriate animal studies in achieving the required evidence. Dr. J. Richard Crout, explaining the provision for controlled studies beyond physician observations or testimonials, declared, "The requirement for adequate and well controlled studies is a demanding one but is essential to rational and consistent decision-making. It is a legal standard because it is a recognized scientific standard." (10) Thus, science directs and the law accepts.

Direct statutory recognition, however, appears in the section of the food and drug law that allows for human studies of investigational new drugs by qualified experts. This usage is exempted from general compliance provisions applying to new drugs when, among other conditions, the applicant submits that "before any clinical testing of a new drug is undertaken, there are reports or preclinical tests (including tests on animals) of such drug adequate to justify the proposed clinical testing." In this case, the FDA has detailed regulations for the various phases of testing, including essential animal studies. So, quite apart from any belief that new or untried drugs may be tested in human beings without prior animal work because other prehuman methods are available or because animals should not be exposed or sacrificed, the law, as interpreted by the FDA as the regulatory agency, has accepted recognized scientific methodology, namely, animal pretestings.

The National Institutes of Health (NIH), which is not a regulatory agency, has effectively instituted prior animal studies through its system of review and approval of applications for biomedical support. The scientific advisors and staff of the agency have uniformly stipulated for scientific, economic, and ethical reasons that appropriate animal studies be associated with clinical research. The most extensive of its testing programs, under the Cancer Institute, proceeds fundamentally on primary toxicological and other studies on mice and other animals. The pervasive approach at NIH, and for its grantees and contractors, is the discovery and application of suitable animal models as a scientific base. Other precursor work is also stressed, as has been mentioned, but both are proceeding at the same time.

In one area, animal studies are prescribed by the Department of Health, Education, and Welfare as part of the program for protection of human subjects: activities in connection with studies on fetuses, pregnant women, and *in vitro* fertilization. Regulations recently issued by the department declare, "No activity . . . may be undertaken unless: (1) Appropriate studies on animals and nonpregnant individuals have been completed." (11)

Thus, there are positive or mandated uses of animals for scientific purposes and also assurances that maximum protection will be afforded human subjects through comprehensive pretests, including animal studies.

Potential Liability

Use of animals may also be implied because there would be liability otherwise. Although medical scientists are less visibly vulnerable than medical doctors to charges of malpractice, they are no less subject to professional liability, essentially on the same basis. Just as malpractice may be alleged when the practitioner has deviated from norms or standards accepted in the professional community, so researchers who perform in substandard fashion may be charged with malresearch. All professionals have an obligation to observe and meet the test of adequate conduct, not superior or even optimum performance. They must use their knowledge, experience, and available resources for responsible and conscientious service. Any deliberate or negligent failure in this regard may be grounds for personal liability. This is the legal, as well as the professional, standard (12; 13a,b).

Significant court determinations rely heavily on the context of the legal issues. Economic, psychosocial, and scientific considerations therefore are brought to bear on interpretations of new statutes and established legal doctrines. For instance, in medical malpractice cases, the well-known locality rule holding that actions of physicians must be evaluated on the basis of current practice of similar colleagues in the same geographic area has been so extended that it is largely superseded. In view of the easy access to modern communication and transportation and the expectation of continuing professional awareness, courts require all members of the professional community or specialty to know and use available resources. The country doctor or the practitioner without modern equipment or support cannot justify a lower standard of care, even if that is prevalent in the immediate area.

So, the clinical investigator and all researchers, whether engaged with animals, laboratory activities, cell studies, systems research, or even statistical and design or surveillance technology, are, by law as well as by professional standards, obligated to keep abreast of new developments and expected to employ accepted practices. This does not mean one standard or one practice, but any followed by a majority or even a respectable minority.

Methodology is regarded as legally sufficient when it has been acknowledged by the scientific community. Thus, use of models, systems, or analogues will be accepted, on a progressive basis, as such techniques achieve recognition. Evidence in law, as in science, has greater or lesser weight, depending on its cogency and relevance to the issues and its scientific probity in support of its intended purpose. A common legal expression, "the best-evidence rule," determines what the courts will admit to support a position. Courts like facts rather than supposition or inference. Therefore direct observation, for example, comes before indirect hearsay statement. But as more complex matters came before the courts and legal tribunals, attempts were made to introduce scientific and technical tests and conclusions. For instance, demonstrative evidence, such as photographs, X rays, and diagrams, were admitted as they proved their reliability. Certain biological tests, blood groupings, and genetic patterns are still subject to argument and are usually admissible on a qualified basis. Thus, the new technologies and methods we have discussed will find legal hospitality as they earn acceptance among scientific bodies.

The investigator is constrained to develop the research design and to follow the procedures that will most likely yield scientifically acceptable results. Injury to patients or subjects that are traceable to research negligence directly or indirectly controlled by the investigator will subject him to potential liability. It is true that physicians and hospitals, because they provide medical service, are most vulnerable; but their liability may result not only from therapeutic, but also from research practice, as in the case of the physician–investigators who used elderly patients in a chronic disease institution to test rejection of injected substances derived from a cancer-cell culture (14).

Beyond personal liability, investigators have the burden of social responsibility to perform precursor studies that will limit the number and risks of human clinical investigations. As Dr. Hermann Blumgart said at the 1967–1968 *Daedalus* meetings, there must be studies in "test tube, in plants, in animals or in other biologic experiments." Adverse effects must first be checked in animals (15). David Cavers, legal expert on the FDA, who also participated in the *Daedalus* conferences, decried the large volume of human experimentation: "There is ample evidence that much of the human experimentation involved in drug testing is wasted." (16) He suggested more careful legal scrutiny of investigational drug practices to cut short human exposures and to reduce or eliminate questionable research, profit-oriented studies, and duplicate efforts involving drugs of small value. The long-sought journals and articles on negative results may well be saviours of human and animal lives as well as of research efforts.

Constructive Use of the Law

How can we use the law for many as a new scientific tool or provision, although long available? Law can authorize or promote; it can control or regulate by statutory interpretation or by decisions in individual cases; and, in an equally valuable sense, it can be constructive by not entering a domain where other measures may be more effective in the public and private interests. In this field, as noted, statutes have been used extensively at local levels to control animal availability and use and also, lately, human experiments. Federal and state laws protect animals and insure care and welfare. In regulating human investigations, statutes, rules, and official guidance indicate when animal and other studies are needed as antecedents to clinical trials. One remaining consideration is how to employ this legal armamentarium and what additional legal assistance should be sought.

I would like to point out, parenthetically, that I think we should have given more attention at this meeting to the self-regulatory systems that the various professions engaged in the use of animals and research have promulgated over the years. Such activity is the hallmark of professionalism, and, to a large extent, this is the way scientists and other professionals are distinguished—by their ability to recognize problems, to police themselves, and to insure that colleagues do the same. In this field, I would hope that we would reinforce self-control before we move to the law. In a rather strange way, perhaps, I am a lawyer who says, "No more law."

Recommendations

Fundamentally, the proper exercise of professional and humane responsibility for human beings and other animals should rest with those most knowledgeable and with the greatest interest in scientific advance, provided they also display concern for all who may be affected by their work. Research investigators, as people, may be expected to share the regard for human and animal life and dignity expressed by the general population and also add their expertise to assuring protection and sensitive compassion.

In the history of science, the integrity of the investigators has been the primary basis for such assurance. When this dedication and obligation has had to be expressed, codes and guidelines for voluntary compliance have been prepared by the profession. The reported success of the voluntary organization of laboratory animal facilities (the American Association for the Accreditation of Laboratory Animal Colleges [AAALAC], founded in 1965 to accredit appropriate centers and facilities, has applications from 470 of potentially 1,800 eligible institutions and has approved 266, housing over half the laboratory animal population) illustrates this possibility.

On the other hand, laws are proposed when voluntary regulation does not succeed. The passage of the National Research Act of 1974 (P.L. 93–348), creating the Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, and enactment of human study and [illegible] research laws at the state level represent the most recent public reactions to presumed excesses or irresponsibilities by scientists.

In a recent editorial, Dr. Maurice Visscher, President of the National Society for Medical Research, reviews a current antiscience movement directed toward full recognition of the rights and interests of the entire animal kingdom. Dr. Visscher urges attention to the publications of this type and their public appeals (17). The implication is that impeding legislation may be proposed unless the scientific community clearly demonstrates the benefits of its work and the justice and fairness of its methods.

Vigilant self-regulation and conscientious observance of existing regulations and guidance should be promoted through education of the public as well as scientific communities and encouragement of effective participation on the part of concerned groups.

In light of the extensive resources now available, no further legislation seems indicated. Instead, such laws as the Animal Welfare Act and the National Research Act and the various scientific and governmental guidelines should be conscientiously followed. Advice and assistance is available and should be publicized. For example, scientists, research institutions, and the public should be aware that the Department of Health, Education, and Welfare provides significant service in meeting animal, as well as human, studies requirements as part of the general review process. The Office of Protection from Research Risks, which is mainly concerned with the administration of human research, also has responsibility for review of protocols or research progress reports to insure that laboratory animals are appropriately selected and effectively used. Such responsibility comes under the Animal Welfare Act and is subject to Department of Agriculture regulations.* This office is now suggesting guidelines or regulations regarding transportation and protection of laboratory animals. If and when approved, animal as well as human participants in research will be further safeguarded.

More important, the public needs to be assured and involved in effective use of animals, as much for educational as for research purposes in schools, museums, zoos, and environmental centers. The participatory process must include lay representatives, and the methods of self-regulatory scientific activity must be explained and illustrated. Congressman Foley also indicated that vigilant self-regulation and observance of existing regulations should be promoted. We can encourage groups here and others to make sure that present laws are followed.

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*See Addendum.

A legal information and advisory service should be established as a jointly sponsored program of all concerned interests and administered by an objective, respected agency such as the National Academy of Sciences' ILAR to serve public and private groups.

Although there seems to be no need for additional legislation, there is clear need for an assembly and exchange center regarding existing national and local laws, regulations, and the application of international agreements, such as the requirements for Certificate of Need papers for procurement of certain animals. The proposed service would not provide the legal counsel that individuals and agencies would wish to obtain through customary legal sources. Rather, the service would maintain texts and references, as well as lists of regulatory and advisory agencies charged with legal surveillance.

I would hope, for example, that some of the comments made earlier today about the involvement of public groups and concerned societies in the review activities at local levels might prove to be adequate. Certainly I think they can be: They can promote laws when needed, and, if not needed, they can show how they can serve science.

The value of such a service in identifying laws, decisions, and opinions at all levels would, at minimum, help to reduce unwitting legal violations and remove many of the delays and frustrations now confronting scientists, suppliers, and review and enforcement bodies, such as the Department of Agriculture, because responsible people are just not aware of legal requirements. The welter of laws and regulations affecting local, state, and interstate, as well as national and international, uses, sources, and conditions for management is baffling. Scientists, their commercial and academic sponsors, suppliers and handlers of animals, review committees, and local police and health agencies need not know all of the legal ramifications, but they should be in a position to get accurate, complete, and current information in this field, just as scientists and physicians can get professional information.

This service could also supply referral assistance and analyses of laws to show uniform and differing local requirements. Such studies might lead to modernization of current laws and removal of inequities and unmanageable provisions. Indeed, such reviews might help reduce conflict between national and state regulations and propose limitation of legal action where voluntary activity seems sufficient.

Finally, such a service would be instrumental in legal liaison—relating these interests to protection of vulnerable and endangered species, to research review committee education, to development of appropriate insurance coverage, and generally to strengthening the man–animal relationships.

As an advisory resource, not a supralegal or administrative agency, a legal exchange can suitably complement the responsible scientific work that has been a primary support of human welfare, health, and education.

Settlement of differences should be achieved, wherever possible, through peer considerations and use of informed, neutral determination, such as arbitration.

There are a great many issues that seem to come to no useful or satisfactory conclusions. If left hanging, these can add to the conflict and to some of the difficulties that seem to confront many of us.

The American Arbitration Association, among other organizations, has been working in the field of prevention and reduction of conflict. Perhaps some of the suggestions that come out of that experience might be useful here. Relevant to our discussion is a quotation from "Scientific Freedom and Responsibility," prepared by John Edsall for the American Association for the Advancement of Science.

He says, in respect to scientific difficulties and disagreements:

> We make no claim to have definite answers to all of these difficult problems. Our own bias would favor resolving difficult issues in favor of human health and environmental quality, as against primarily economic considerations; but we would most urgently plead for increased foresight to anticipate such dangers at an early stage, before large-scale industrial development occurs, so that these grim conflicts between opposing interests may be avoided as far as possible.

He then notes that it must be possible for scientists who may disagree with their employers to speak up, and notes that a declaration, "Employment Guidelines," has been endorsed by scientific and engineering societies. He quotes from it:

> The professional employee should have due regard for the safety, life, and health of the public and fellow employees in all work for which he/she is responsible. Where the technical adequacy of a process or a product is involved, he/she should protect the public and his employer by withholding of plans that do not meet acceptable professional standards, and by presenting clearly the consequences to be expected if his/her professional judgment is not followed.

Many scientists, however, have got into trouble following just this tenet or principle. Dr. Edsall then suggests:

> that the formulation of such a declaration is a significant event. But it will have meaning only if it is effectively applied. . . . These guidelines, like most such codes of ethics that we have seen, lack a very important ingredient, namely, a provision for the arbitration of disputes. The protection of individuals from arbitrary action by authority is deeply ingrained in English common law, and the U.S. Constitution provides that "no person

> shall be deprived of life, liberty, or property without due process of law." . . . We believe that some form of due process should be an essential part of any employer–employee agreement or contract to protect the employee from arbitrary action by the employer, allegedly based on professional or personal misconduct. A minimum requirement for such due process would involve a hearing by a board, including independent members. . . . (18)

In effect, this is an arbitration proceeding. At this stage, it would not go to the public courts. It would not be in the headlines of every newspaper. It would be handled as a part of a peer review system, in which panels of the public, from your own profession, from those also concerned, might privately, but effectively, be involved to make decisions. In some cases, court review would be needed.

Perhaps, therefore, before conflicts become conflagrations, you may wish to think of simple ways to put out the little fires.

ADDENDUM

Public Law 94-279, 94th Congress, April 22, 1976, amended the Animal Welfare Act of 1966, as amended, to increase the protection afforded animals in transit and to assure humane treatment of certain animals and for other purposes. Included in this legislation are authority and direction for the Secretary of Agriculture to prescribe rules relating to: reporting and inspections, health certification on animals being shipped, minimum age for animals being shipped, financial arrangements with carriers by person shipping animals COD, and prohibition of animal-fighting ventures. It also clarifies the application of the law to governmental laboratories and research and defines the interstate jurisdiction. It may be noted that many states have similar requirements for inspection, reporting, and care of laboratory animals.

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DISCUSSION

TREAT: Some say that you cannot legislate morality, and that is a good point, but the abolition of slavery is one example. If someone could enlighten me as to whether these animals are anesthetized, I would appreciate it. But my first two points are about Dr. Pfeiffer's comment on the use of prisoners and about why research grants are so allotted that a researcher is actually not encouraged to quickly complete his assignment. I am not saying the man shouldn't be given enough time, but that he is not encouraged to complete it quickly.

LADIMER: I worked at NIH. The administrative aspect of grants is not a legislative matter. As to the return of funds and checking on research, any granting agency—NIH is by no means unique in this respect—is obligated to find out what happens to its money. I don't think that is unconscionable. If a person finishes the research work intended beforehand, he is obviously expected to explain it. If there is any money left over, he should return it, so that others may have a chance.

By the same token, if he is doing a good job and hasn't finished and needs more, perhaps he can get it. He may even get more animals for future work he has planned. I don't know that there are any figures or other data on whether this practice has encouraged or discouraged people from doing good work. Perhaps the unused money is available to buy file cabinets or increase the number of secretaries.

I don't believe that this feature presents a serious issue in respect to the overuse of animals. There are other apsects of greater importance. This is basically an attempt at improving accountability, namely, any organization that gives contracts or grants has a right to know how its funds and resources are being used.

As to prisoners, I was rather surprised to hear you suggest what you did. That suggestion is centuries old. It is recorded that in the thirteenth century prisoners were used for experimentation and vivisection. Since that time, there have been suggestions at various times to do that again. I would say in every respect, and at all times, the medical profession has refused to become an executioner. If this is a field in which you feel prisoners should be punished, the medical profession does not want to be part of the axe.

TREAT: With informed consent.

LADIMER: Informed consent has nothing to do with offering death by law or death by science. This is not to say that prisoners should be prohibited from true voluntary participation. It is a question of whether or not prisoners who are incarcerated for a particular purpose would be served in any way by informed consent to become subjects. But there should be no question of the condemned prisoner serving as a sacrificial lamb. I think informed consent is entirely possible, but not because these confined people are coerced or convinced to provide a final altruistic gesture to avoid getting the axe. I don't see that this is a choice, if you are talking about informed consent.

There are ways in which prisoners can effectively be used—and I have worked on some of these ways—but not on that basis.

On the pain issue, I am certain that there are many here who would agree with you completely. I just don't know enough about animal pain to be able to measure it in this way. I assume that they feel pain, as Dr. Rowsell pointed out. Therefore, I am sure such studies are not undertaken lightly.

LORD: Dr. Ladimer made a plea for self-regulation, and I have noted that really nothing has been said about the activities of AAALAC. For those of you who don't know, this organization was formed, I think, 10 or 12 years ago, and it was an attempt on the part of the scientific community to regulate itself in reference to animal care and husbandry.

I think it has been successful to a degree. It has been successful in the sense that those institutions that needed funding to do a better job in their facilities were able to get it.

I know that I personally have been on site visits wherein the conditions were such that we withheld accreditation and as a consequence the institution was improved, and I think this symposium should at least reflect the success, small as it may be, of AAALAC.

LADIMER: Thank you for mentioning that. The AAALAC, as well as other organizations, has done a highly creditable job. I firmly feel that the use of the accreditation process on a private or informal basis or a voluntary self-regulatory basis has a great deal to commend it.

COOKE: I would like to ask, Dr. Ladimer, if you have case histories supporting your interpretation of the law that would suggest that malpractice suits would be forthcoming if researchers did not use animals.

LADIMER: Not as such. There have been cases when the lack of animal data or use of human beings without adequate prehistory have been included as part of the charge of malpractice. For instance, the failure to examine the animal data sufficiently was noted in the thalidomide and MER-29 cases. There have been cases against researchers for other types of deviation from accepted practice. This is an analogy that I have suggested because of the animal-test guidelines now required.

I do know that where the animal studies described in project applications have not been considered sufficient—sometimes sufficiency does not mean quantity but may mean quality—investigators have not been able to get contracts or grants. That is far from malpractice, to be sure, but it does present a limitation. This current practice does not mean there can be no change. But at the present time, use of animals is one of the usual specifications.

COOKE: If I understand, the answer to my specific question is no.

LADIMER: That is correct. But, my commentary is important.

BRAUN: I have had concerns, and I wonder how the medical profession has resolved this problem. In the course of medical training, the medical student has got to desensitize himself in order to be able to do animal experiments. Later on, he has to change hats and resensitize himself to treat human beings with compassion. I am wondering if it is possible to resolve a dilemma like that, and how it is resolved in actual practice.

The other question I have: Can you conceive of the law serving to protect animals as well as human beings? In other words, can you conceive a legal group trying to work out what are the legal rights of animals who are completely under our power and control?

LADIMER: I think there is an implication that medical students are cruel to animals and are kind to people. I am not sure that is right, and so I am not sure that the problem of a switch is all that great. They may be in reverse, but, in any event, there is nothing that I know of in medical schools—I have been to a great many of them—in which the use of animals or the observation of animals are suggesting that use of animals is anything less than kind or compassionate. If it is not, it certainly can be made so. Treating living creatures is really part of a whole system, not necessarily two separate systems in which you move from one to the other.

In answer to your second question, protective laws already exist. There are a great many provisions of concern for animals. Whether they can be equated as being of the same quality or kind as for people, I can't say. A major problem, of course, is that you can't talk to animals. At least I haven't been able to. So, you can't ask them the kinds of questions that presumably informed consent requirements provide or permit us to ask of people. We have to infer a great deal, and to that extent I believe we have a great many organizations and statutes that have made such inferences and have suggested how animals shall be recruited, cared for, treated, or put away if necessary. The answer to your question about laws for humane treatment definitely is yes. Laws can be passed for this purpose and have, in fact, been passed.

HOWARD A. SCHNEIDER

Summary

I think I must begin with a few observations that are made necessary by the special, even experimental, nature of this conference. This meeting is, in a sense, a kind of ecumenical assemblage, for here have been brought together working scientists with a special expertise in the use of animals in scientific experimentation and a laity concerned with the humaneness with which these scientific enterprises are conducted, and indeed their very necessity. It is highly probable that some semantic problems may hamper my efforts to explicate matters, but like everything else, a beginning must be made somewhere. I suspect that this conference, in perspective, will be seen as just such a beginning.

There is a second preliminary observation that I find necessary because I feel that it has been left unarticulated, but it is a silent and important presupposition that, if examined, can be seen to add to our understanding of why we find ourselves doing some things as scientists, what nonscientists sometimes find difficult to understand, as part of the scientist's business. I refer to the very real consequences of a historical fact. It is simply the fact that Western science, in one of whose halls we now sit, is a human enterprise of but the last 300–400 years, and it arose and still flourishes in a setting dominated by the ethical outlook of the Judeo–Christian tradition. Let me repeat this for emphasis. Applied science is a product of the West. It arose in the Judeo–Christian world of the West. That stark fact has consequences for us here today, as we shall see.

Earlier I identified two components, scientists and laity, of th conference membership. I think I should remark on a third component who joined us, a member of the Congress of the United States who has a special legislative interest in the concerns of this meeting and who is, in a sense, removed from both scientists and the laity. I refer to the participation here of the distinguished chairman of the House Committee on Agriculture, Mr. Thomas S. Foley. Although he appeared here single-handed, he brought, I think, an important insight. But the presence of Congressman Foley has, I think, drawn attention to still another unarticulated presupposition that merits the attention of this conference.

We are all aware, of course, that for some time the field of use of laboratory animals has been regulated in the United States by the Laboratory Animal Welfare Act of 1966, as amended by the Animal Welfare Act of 1970. The hearings held before the Congress on these legislative acts, and in which I participated, instructed me in the very real philosophical differences between the processes of truth-seeking, which exists in a legislative body dominated by the legal process as is the Congress, and the processes that have become ingrained in the professional activities of scientists (1). Truth-seeking in the law begins with the presupposition that one must provide a fair forum for antagonists and protagonists. In the interplay between these two polarized groups, the truth will be found. This leads naturally to the evocation of compromise. The truth is rarely found at one pole or the other. It lies somewhere in between.

Now, the scientist does not proceed that way. When he finds himself in the lawyer's forum of advocacy and counteradvocacy, he sometimes fumbles. He feels ill equipped, but he has begun to learn this new terrain, because that is where now, increasingly, his fortunes are being decided. On the other hand, scientific truth-seeking begins with a flight of the imagination into a multidimensional world. The scientist ideally makes no firm presupposition where the truth is at the start and is moved to probe and try and try again. Gradually the scientist comes into focus with something that he can put into some manageable system. This he struggles to improve in his approach to a publicly verifiable "truth." This view of truth-seeking in science has all been summarized in a beautiful book by Sir Peter Medawar, a Nobel Prize winner in medicine and physiology, entitled *The Art of the Soluble* (2). If politics is the art of the possible, science is the art of the soluble.

I hope my scientific colleagues will be understanding if I am somewhat cavalier with their contributions. I cannot use here exclusively the precise words, and jargon, of science that would so easily improve and shorten my communication. It will be necessary, I think, to use the words of common discourse. In such discourse one uses the more ordinary words of our speech, and that, too, may be a test of our hopes of communication here.

The historical perspective of biomedical experimentation, as given by Dr. Davison, was a heartwarming recital of biomedical history. One felt, as a scientist, proud to be a member of that company.

I believe that Dr. Davison touched an important note when he said:

> I am left with the final conclusion that to abolish the use of animals in teaching and research would fly in the face of the lessons of history and be far more inhumane to society, both man and animals, than to encourage their reasonable, humane, and justifiable use.

Now, that is the standpoint of the historical perspective, but we stand here now asking another kind of question. Can we change? Can we now make use of technologies, of techniques, that were not available then? And that is the root of this whole symposium, is it not? It is to examine now in this present scientific context, with scientists rendering their judgments, as to whether or not we can get on with our job by utilizing other systems, other means, computers, inanimate models, and so forth.

The humane perspective, as given in Mrs. Christine Stevens' and Dr. Hummer's presentations, showed that the humane community in this case certainly is not monolithic, no more than is the scientific community always of a single mind. I was delighted that Mrs. Stevens introduced Darwin. Darwin is a historical favorite of mine, and I am going to allude to him again later.

The presentation of "humane perspectives" are, however, joined perhaps in a subtly pejorative way, for it has always puzzled me why scientists should, without further ado, and by exclusion, be consigned beyond the pale as inhumane and be assigned other places on the program. But we need not press this matter, which is more a matter of imagery than a deep philosophical meaning. Further beneath the surface, however, we come upon a more fundamental matter. The humane movements were born in a historical context when the work performed for human ends was in large part done by the muscles of animals, and visibly so, even in the cities. These needs, and their rightfully abhorred abuses, have now in large part been fulfilled by the energy derived from the combustion of fossil fuels. But if animals are now found in other roles, the same philosophical question remains, "Is it proper that animals be used for ends defined by man?" We can rephrase that question, more narrowly for the purpose here, "Is it proper that animals be used for scientific ends defined by man?" This is an ethical question, and for science, we can turn to a public formulation of the ethical position as sanctioned by the relevant articulated consensus. I quote from Baker's *Dictionary of Christian Ethics*, which was edited by Carl H. F. Henry Bakes and was published in 1973. Professor Smick, who was professor of systematic theology at the Reformed Theological Seminary, in discussing animals said:

> While animals and all life are to be respected, it is never at the expense of man. If rats need to be destroyed for human good, so be it. . . . The extreme reverence for all forms of life in the oriental religions is out of balance. A distinction must be made between killing and cruelty. (3)

In the next section of the symposium we heard of problems that biological scientists have wrestled with a long time. This world, alas, is not a neat and tidy place. It is filled with variation. We are indebted to Dr. Race for his scholarly review, and I was particularly struck by his reminding us that the very variation of the biological world, in evolution, provided for an emergence, as we moved up from lower levels, of new qualities that were not present in the lower ones.

Because of that evolution in its profusión, various choices and experimental options are available to us. Dr. Race pointed out, for example, that in addition to the simple whole animal, special inbreds and hybrids were available, as well as cell cultures, organ cultures, and such scientific derivatives as computer assisted research, pharmacogenetics, and the extensive biological variability of the immune response. Variation is both a challenge and an opportunity.

The various experimental systems, advantages and disadvantages, were reviewed by Dr. Kurt Benirschke. He addressed himself to four questions and their answers and said: "Are there valid reasons why certain experimentation requires intact animals, specific species, or developmental stages?" He answered that question with an unequivocal yes. In doing so, he dealt with such matters as leprosy and chimerism and inborn errors of development. He concluded there was no escape from intact animals in these fields.

A second question posed by Dr. Benirschke was, "Can experiments of nature be depended upon and how can we recognize and follow through on them?" In sum, Dr. Benirschke, who was seconded by Dr. Leo Bustad who spoke later, found these "experiments of nature" a rich source of insights into the world of animal life.

In his third question, Dr. Benirschke recognized the lack of standardization of such variables as nutritional or environmental status as possibly affecting the reliability of intact animal experimentation. Today we are fairly in the vineyard of the Institute of Laboratory

Animal Resources and the mission that organization embarked upon a long time ago. As Dr. Benirschke remarked, "At least for the common laboratory animals, standards of care and housing, management, and diet have been set to minimize these variables." And I think I will be permitted to add that in recent years we have seen a quantum jump in the improved definition and control of these important variables in biomedical research, and, although it has not been mentioned, I think the scientific community can take pride in the fact that it pioneered the whole development of a system of gnotobiosis, in which animals, after Caesarian section, were taken into barrier-sustained quarters and had a package of known microorganisms given them. One long-troubling variable, the animal's microflora, was under control to a degree theretofore nonexistent.

Finally, Dr. Benirschke questioned, " . . . when alternatives exist, that is, culture methods instead of animals for the study of disease, do these alternatives have an equivalence to information from intact animals?" I think this has been repeated time and time again, and I will not dwell on it further, only to say, with Dr. Benirschke, that these alternatives are complementary, but do not displace the need for animals.

Why are these alternatives complementary, and why do the scientists want to use them? This goes back to Dr. Medawar's analysis of scientific progress, of the next step, of the solution of what is soluble. It is that by reductionism, by being able to extract a question, a problem, from a very complex array, that one has an increasingly manageable way of coping with it. Something has been lost in the extraction, however, and so the path must be retraced eventually in order to find meaning, but, at the moment, the scientist can only do what he can do. He cannot wait for the rest of his life for the solution of some grand problem. He must do what is in front of him.

The symposium next took up the problems of animal behavior studies. It was very clear that here was an aspect of biology for which there was no imaginable substitute. The simple truth is that only an intact animal can have behavior.

Again, we are driven to experiments on intact animals once we are committed to a search for such knowledge.

I think it is at this point, in discussing commitment, that I would like to introduce my hero, Charles Darwin. I was much upset when one day I learned in a publication that Darwin had said that he could not sleep at night for thinking about animal experimentation. I had collected the works of Darwin, and I have read many of them. This purported quotation did not agree with my understanding of these matters, and so

I turned to my two-volume edition of *The Life and Letters of Charles Darwin*, by his son, Francis.

I found Darwin writing to Professor Ray Lancaster, on March 22, 1871, "So I will not say another word about it, or else I shall not sleep tonight." But what did he say just ahead of these words? He said, "You asked my opinion on vivisection. I quite agree that it is justifiable for real investigations on physiology, but not for mere damnable and detestable curiosity." (4)

That puts matters in a different light. But in another quotation it is clearer still. In 1881, Charles Darwin wrote to a professor of physiology at Upsala:

> On the other hand, I know that physiology cannot possibly progress except by means of experiments on living animals, and I feel the deepest conviction that he who retards the progress of physiology commits a crime against mankind. (5)

On the relationship of animal behavior to illness and disease, how grand it was to hear that there was a medical school (at Hershey, Pennsylvania) in which medical students were now being instructed in the principles of behavior, and appropriate teaching tools were there arranged by means of observation, under suitable conditions, of animal life. I can do nothing but applaud this pioneering effort, and I think that, again, there is no substitute. Animals do have, of course, the advantage for us of a shorter life span, and to teach medical students the comparisons between animals and men can be nothing but, I would insist, "humanizing."

Professor Bustad told us a good deal about animal models of the human condition, which I cannot condense here. The list is extraordinarily long. I have read this manuscript, and I was absolutely staggered by the size of the bibliography. The references numbered over 6,000 and were cut to 800 for practical purposes of publication.* Dr. Bustad deserves our special thanks for a labor of love.

In the matter of computer simulation, I think we had a grand exposition, a realistic description of what computers can do, and a deflating of the computer mystique, for there is indeed an unnecessary mystique about computers. We have been told they can do wonderful things. Actually they are morons, and can respond only to instructions, meticulously programmed into them. The reason why computers are nonetheless valuable is that in compilations of statistics—and this was quite correctly pointed out—there is presented a large, almost unmanageable welter of data that somehow must be put into some kind of shape that one can then contemplate and manipulate. Here is a clear instance of the merits of a tool for research, but we must not be misled into forgetting that the hammer that drives the nail does so while held by a human hand.

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* See "Animal Models" by Leo K. Bustad *et al.*, pp. 130–151.

The computer *cannot* make a discovery. A discovery is a flight of intuition. A computer has no intuition. It only has in it what you put into it, and it will do it marvelously well, with no fatigue and no errors. And so, as Dr. Carol Newton rightfully pointed out, it can do so many useful things for us. It can make us aware of some things that we previously were unaware of, but will need testing. It will suggest things that have escaped our notice, because they were so massive, so complex. It will simplify them for us, but we will have to test the concepts in the end.

I think we should heed Congressman Foley's words, for he is an experienced legislator. He felt that we were not now under any gun and there was no need now for more legislation in the laboratory animal field, but what was even more significant, I believe, followed this announcement. It was that the Congress and its committees focus on problems with reluctance after matters get heated up by controversy.

If, on the other hand, problems are solved by consensus between the interested parties but may need some appropriate and formalizing legislation, Congress is very receptive to such a need, to something that is "presolved," is appropriately stated, and the necessary information is supplied. But contrarily, when there are contention and adversary relationships, the congressional committees are reluctant to sit as ajudicators, especially, as I observed earlier, because of congressional sensitivity to the antagonist–protagonist relationship.

I think we should ponder Congressman Foley's advice, and I think and hope that in the dialogue that we begin today—tortured though it is, incomplete though it is—that we remember this advice and that we begin offstage, outside of the arena of contention and adversary relationships, a dialogue that can be productive. I think that this would be the grandest kind of outcome this conference could possibly have.

Next, the past, present, and future of *in vitro* systems were discussed by Dr. Hsu. He alluded to the example of the fantastic impact of the discovery by Enders that he could grow many viruses, polio viruses included, in monkey kidney cells in tissue culture. That changed things overnight, and I would emphasize that the use of animals in that area of medical research practically declined to the vanishing point, compared to the numbers that were formerly used in such large quantities in order to titrate the viruses. This was an absolutely overwhelming development.

The rediscovery of the egg as a culture medium for certain viruses is another example of the automatic adoption of suitable systems for infectious disease when they can be found and the subsequent reduction in the use of animals.

It was in the use of overt *in vitro* systems of tissue culture in basic biomedical research, described by Dr. Mary Dawson, that I thought we had a clear signal that the role of such tissue cultures was complementary to animal use and that, as she said, there is undoubtedly scope for a much wider use of tissue-culture methods to add to our imperfect knowledge of many diverse aspects of biomedical research.

However, if tissue-culture tests are to be considered not as adjuncts to, but as replacements for, some existing whole-animal test, then much more work needs to be done. That replacement does not seem imminent in the foreseeable future.

Dr. Federoff's discussion of the use of *in vitro* systems in medical research was, I thought, a magnificent *tour de force*. His was an exciting presentation, and I just hope that the audience caught that excitement and some of its promise, however remote. He, too, felt that the study of tissues in isolation departed in a significant way from a whole system and lost the effect of participation in the true biological system of an intact animal, in which there are a multiplicity of feedback mechanisms.

Our deplorable hubris in these affairs springs from the fact that as we proceed in reductionism we gain mastery, and we think somehow that we are mastering the event that we now have abstracted and simplified until we can understand it. But what lies behind us, and which we must now retrace, are the steps back up into the intact system. Here complexity again lies in wait for us, and our real progress lies operationally in accommodating complexity in our strategies in the first place.

The application of *in vitro* systems to public health, the use of cells in vaccine production, was well documented by Dr. Petricciani. The use of cultured cells in drug screening and in the difficult problem of detecting mutagenicity and carcinogenicity has a useful potential, but is not a solved problem. This circumstance seemed also to exist for Dr. Loomis, who led us by the hand through the details of what it means to come to some hard and firm data in a biological system addressed to forming an estimate of the efficacy and toxicity of a useful chemical. In passing, he asked a very meaningful question. He asked, in the situation of animals versus man, "What is expendable?" That is a very fundamental question indeed. What *is* expendable? The gaining of knowledge exacts a price, and, in increasing our knowledge of human life, we have drawn back from expending, knowingly, human life itself. Animals, on the record, are expendable; humans are not.

Now, what is our humane duty? Humane duty to whom? What is the presupposition? The presupposition, you see, will have to acknowlege that fundamental ethical commitment to the Judeo–Christian tradition, and in that it follows that man is above the animals.

I recognize, admit, and fully entertain the idea of responsibility, and I think that the interesting survey of the Canadian biomedical research community that was provided to us also showed us that there was no turning back. There is no retreat. There is no way of gaining innocence. We must do what we can with what we have.

I think that there was in Dr. Rowsell's presentation on ethics a forthright listing of the points of tension, and I don't think it would be right for us to expect him to fully resolve these points of tension. I think we owe him a debt for having clearly delineated what those points of tension were. In Canada apparently, ethical problems are best handled at the institutional level.

In the presentation of ecological considerations in animal usage, I was most pleased, for much of the basic data came from the collections and hard work of the Institute of Laboratory Animal Resources. But the conclusion was, I found, a depressing one. That was that we are, as in so many matters, pressing against our finite resources on the globe.

The questions as to whether or not wild animal genotypes represent a renewable resource I think is worth looking at. It was under that heading that Dr. Talbot gave us his recommendations for a lowering of demand. Research for the survival of stocks seems to be an area in which, in contrast to supplies of petroleum, which will run out in about 35 years, wildlife has prospects of continuing, if we all now realize the threats to it and move to support such research.

The legal aspects of biomedical experimentation were really told the way it is, and I think we are fortunate to have Dr. Ladimer's exposition. That is the first time I have ever heard a delineation of what the law says, and I think we must congratulate Dr. Ladimer for sharing his experience with us. I didn't know, and I now must ponder the fact that, the FDA actually has legal standards because they are scientific standards. That puts the matter in perspective in a new way for me; and, as well, that the Department of Health, Education, and Welfare firmly mandates animal studies before studies on fetuses and pregnant women can be pursued.

The plea for self-policing by the profession, instead of a smothering layer of regulation and law, is one with which I totally agree.

Finally, in these many alternative systems that we have discussed in relationship to animal experimentation, I conclude that to varying degrees they have a place in biomedicine; they are complementary and help fulfill our dreams of reductionism, but they will not replace animals, not yet. They have a place, but they will not replace.

The probing spirit of man bristles when, for whatever reason, limits to the quest for knowledge are put forward in whatever guise. Galileo finished his life under house arrest, but we see now what a futile exercise in the attempts to restrict an idea that was. But for those who look to the day when animals are no longer needed for the advance of man's knowledge of life—and that day certainly lies beyond the lifetimes of this audience—let us remember that, as history has shown, there are in science itself forces that lead, but slowly, toward that day. It is the inner-directed forces of science that will continue the process of abstraction from *in vivo* to *in vitro*. But the history of science also shows that all this will move at its own historical pace. We must be patient.

Toward this perspective Darwin made a contribution, and I think perhaps it was his most important message. He taught us that we were suspended in a web of life. We cannot escape. We abstract features from it, and our conceits lead us on, but in the end meaning escapes us. The last reduction of the last molecule to the last electron in its final spin leaves us but contemplation. Contemplation of what? We might as well contemplate our navels.

No, we must return to the web, to the matrix, to life itself. One of the lessons of ecology is that everything is related to everything else, and I ask, how do we relate as men—not as angels—and as animals ourselves? Here we are. The stars and the planets will not have us. They are hostile. It is earth that is our only home, and here we are all brethren.

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GEORGE T. HARRELL

Closing Comments

On behalf of the Organizing Committee, I would like to thank you all for coming and participating in this symposium. We would like to thank the National Academy of Sciences for its foresight in recognizing the need for a conference on such an important subject. It has been our privilege to develop the program working with the staff of ILAR. We would like to thank you, the audience, for your active part in the discussions and frank exposition of viewpoints—scientific, intellectually humane, and often purely emotional. I should like to take the liberty, on behalf of the audience as well as the committee, to thank the speakers for the careful and thoughtful preparation of their presentations. The formal papers and transcribed discussions, both by the designated summarizers and the spontaneous reactors, will be published.

The charge to the Organizing Committee was to look at the past 20 years and more in the efforts of the scientific community to help improve the quality of experimental animals, materials, and facilities and the effective utilization of resources in wise, humane, and economic fashion. From this perspective and in the light of scientific advances in this remarkably productive era of biomedical research, the committee was to make an objective assessment of the future of animals and the alternatives available to scientists in research design and education. With this in mind the symposium was designed to examine the past and future use of animals and the possible role and application of cells, models, and systems as substitutes, if suitable, for intact animals. Divergent views were expected and certainly have been expressed. A particular objective was to insure that the feelings and expectations of concerned lay groups and the general public were made known and carefully examined in relation to scientific need and technical requirements.

One of the most useful results of the symposium has been the dialogue that has begun between scientists working at the laboratory bench, administrators, legislators, lawyers, and lay spokesmen concerned about animal welfare and protection. This presentation of differing points of view should be continued, and the NRC–NAS should find a mechanism periodically to provide a forum for discussion to assess new advances in knowledge so that all parties could reevaluate and adjust their positions in the best interests of scientific research, education, and animal welfare. Future basic research will uncover new tools and techniques, further develop and hone existing ones, and apply new knowledge to the improvement of both human and animal health.

The conclusion that seems to stand out most sharply from the 2 days of discussion is that biologic problems are never as simple and uncomplicated as they first seem. Great progress has been made in the replacement of living whole animals by the substitution of fertile chick egg or tissue cultures in studies on the growth of viruses, for example. Progress has been made in the reduction of the number of animals needed for valid results and in the refinement of techniques used to be sure the least distress is caused to animals. The surprisingly extensive bibliography collected on animal models that simulate human diseases is so outstanding it deserves publication in its own right and not solely as references to the one paper. Further, recognition of the existence of such models may reduce the need to explore in multiple species the basic mechanisms of human chronic illnesses that are still unknown.

The further and more intense application of computer techniques in the design of experiments and the evaluation of data biostatistically could lead to future reductions in the number of animals required for a given experiment. Understanding of biologic variability is necessary to achieve the desired precision in research and will affect the number of animals used. On occasion, though, it may increase the number needed. Reduction in the number of variables, by provision of animals well cared for by trained personnel under the direction of veterinarians in as nearly ideal facilities and environment as can be built, will produce better, more dependable data. Such refinements cost more than most investigators and legislators recognize, but they can reduce the number of animals and the necessity for repetition of inconclusive experiments. The suggestion of more complete reporting of the details of experimental design may reduce the number of experiments repeated and hence the number of animals used.

The behavioral scientists have pointed out the absolute necessity for using intact animals in studies that involve the central nervous system and the reaction of the whole body to environment and stress. No substitute is likely ever to be found in view of the complexity of feedback mechanisms and the integrative capacity of the brain. Studies of animal behavior in natural or simulated controlled conditions will help us to understand human reactions as reflected in biochemical and physiological disturbances. Studies of inborn defects in metabolism or development, which are genetically transmitted, by their nature require intact animals. *In vitro* studies of specific enzyme and chromosome abnormalities complement and extend the knowledge gained. Studies on the possibilities of transplantation are best done in identical twins, and several species offer suitable models, but require intact animals. In spite of the spectacular progress made in the understanding and control of infectious diseases, some bacteria and other disease agents still cannot be grown outside of an intact animal. This was documented by the failure of *in vitro* cultures including live cells to grow leprosy bacilli. As was pointed out, the armadillo is the only known source beside the human being for this culture.

The very detailed and illuminating discussions by the cell biologists have demonstrated the great progress made in recent years with cultures of cells, tissues, and even whole organs. The unmistakable conclusion that must be reached from the deliberations of this symposium is that these techniques cannot replace experiments with whole animals, but they give invaluable supplemental information and complement other data collected. The way was pointed out for greater use of *in vitro* techniques as the scope of their possibilities is explored further and, most importantly, compared with results obtained in intact animals.

The importance of a humane perspective on the use of animals was brought out by many lay discussants of the scientific presentations. Speakers repeatedly mentioned the continuing rise in the cost of obtaining, breeding, and maintaining animals. It has not yet been shown that *in vitro* techniques that require meticulous observance of strict laboratory procedures by highly trained personnel are any cheaper than is the good care of animals. The concern of scientists with conservation of animals and endangered species that are being depleted by encroachment of civilization, as well as use in research, repeatedly was brought out by the speakers. This concern was forcefully emphasized in the case of primates. It was distressing to learn of the large number that die in the process of capture and transportation before they reach scientists. It was suggested that the establishment of breeding colonies would alleviate this loss, but the experience with marmosets in captivity shows this solution is not easy to achieve in all species.

It was heartening to hear that adequate legal measures already are on the books and that new legislation should be considered only after careful examination of the facts presented to indicate need. It was suggested that changes in regulations and rules, which are much easier and quicker to implement than new laws, could correct what many lay people see as current abuses in the use of animals.

It is apparent that all of the questions posed at the beginning of this symposium have not been answered fully. Good will, continued concern and discussion, and more and better research over the years will provide additional answers. Science has come far in a very short time, but even more unknown areas lie ahead to be explored and hopefully understood with findings adopted for the better health care of mankind.

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